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THE UNIVERSITY OF ALBERTA

Synthesis of Fluorinated and Phosphono Diaminopimelic Acid (DAP) Isomers as Inhibitors of DAP Enzymes

by

Yonghong Song

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

Department of Chemistry

EDMONTON, ALBERTA
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Synthesis of Fluorinated and Phosphono Diaminopimelic Acid (DAP) Isomers as Inhibitors of DAP Enzymes submitted by Yonghong Song in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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To my parents and my wife, Xiaojie

Abstract

Two hydroxylated and four fluorinated analogues of diaminopimelic acid (DAP) were synthesized in stereochemically pure form. The hydroxylated DAP analogues (2S,3R,6S)-2,6-diamino-3-hydroxydiaminopimelic acid (**29**) and its 2R,3S,6S isomer (**26**), were synthesized by Seebach condensation of (2S)-1-benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinone (**23**) and its enantiomer (**22**), respectively, with (S)-3-(benzyloxycarbonyl)-4-(3-oxopropyl)-5-oxazolidinone (**15**). The 3-fluoro analogues of DAP, the 2S,3R,6S isomer **56** and the 2S,3S,6S isomer **57** were synthesized by Schoellkopf aldol condensation of (3S)-3,6-dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazine **42** with aldehyde **15**, followed by fluorination with (diethylamino)sulfur trifluoride and subsequent hydrolytic deprotection. The 2R,3S,6S isomer **54** and the 2R,3R,6S isomer **55**, were generated analogously from the enantiomer of **42** and **15**. Approaches to synthesis of β,β-difluoro DAP were also investigated. The hydroxylated DAP isomers **26** and **29** were very weak inhibitors of diaminopimelate epimerase (50% inhibition at 2.5 and 4.0 mM, respectively); however, all four fluoro DAP analogues were potent competitive inhibitors of DAP epimerase (IC_{50} 4-25 μM). Compounds **55** and **56**, having a 3R configuration at the carbon bearing fluorine, undergo rapid epimerase-catalyzed elimination of hydrogen fluoride without detectable epimerization at C-2 to eventually form tetrahydrodipicolinic acid (**116**). In contrast, the enzyme epimerizes **54** and **57** extensively at C-2 before slow elimination of hydrogen fluoride occurs.

Four phosphono DAP isomers and their L-alanine dipeptides were synthesized in stereochemically pure form. Of them, (1R,5S)-1,5-diamino-5-carboxypentylphosphonic acid (**92**) and its 1S,5S isomer **93** were prepared from the alkylation of diethyl (1S,4R)-(1,7,7-trimethylbicyclo[2.2.1]hept-2-ylideneamino)methylphosphonate (**80**) with (2S,5S)-1-benzoyl-2-(1,1-dimethylethyl)-3-methyl-5-(3-bromopropyl)-4-imidazolidinone (**85**),

followed by separation of the two diastereomeric products by chromatography and subsequent hydrolytic deprotection. The 1R,5R isomer **94** and 1S,5R isomer **95** were analogously synthesized from the reaction of **80** with bromide **84** (the enantiomer of **85**). These phosphono DAP isomers **92, 93, 94** and **95** were coupled with L-alanine to give dipeptides **100, 101, 103,** and **102**, respectively. The DAP diphosphonate analogue **98** was also synthesized as a mixture of all diastereoisomers. Compound **93** is a moderate inhibitor of DAP decarboxylase whereas **92, 94, 95, 98** exhibit very weak inhibition. Compounds **92, 93, 95,** and **98** are weak competitive inhibitors of DAP epimerase. Dipeptides **100, 101, 102,** and **103** show negligible or low antibiotic activities.

A new stereocontrolled synthesis of DAP isomers has been devised. The reaction of bislactim **42** with **85** gives the alkylated product **107**, which upon hydrolysis in 6N hydrochloric acid affords *meso*-DAP (**108**). The bislactim ring in the intermediate **107** can also be selectively deprotected in 0.25 N hydrochloric acid to produce **109**.

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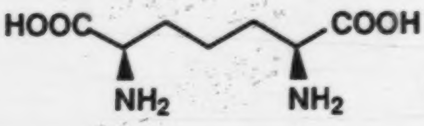
List of Abbreviations

Ac	acetyl
Ala	alanine
Ala-P	aminomethylphosphonic acid
aq	aqueous
Ar	aromatic
AZIDAP	2-(4-amino-4-carboxybutyl)-2-aziridine-carboxylic acid
Boc	*tert*-butoxycarbonyl
Bu	butyl
iBu	isobutyl
cat.	catalytic
Cbz	benzyloxycarbonyl
CI	chemical ionization
CoA—	coenzyme A
DAP	diaminopimelic acid
DAST	(diethylamino)sulfur trifluoride
d.e.	diastereomeric excess
DMF	N, N-dimethylformamide
DMSO	dimethylsulfoxide
Et	ethyl
Enz	enzyme
FAB	fast-atom bombardment
GlcNAc	N-acetylglucosamine
Glu	glutamic acid
HPLC	high performance liquid chromatography
IC_{50}	concentration at 50% inhibition

IR	infrared spectroscopy
LDA	lithium diisopropylamide
Lys	lysine
Me	methyl
MPLC	medium pressure liquid chromatography
MS	mass spectroscopy
MurNAc	N-acetylmuramic acid
Mw	molecular weight
NADPH	nicotinamide adenine dinucleotide phosphate reduced form
NBS	N-bromosuccinimide
NCS	N-chlorosuccinimide
NMR	nuclear magnetic resonance
p	pentuplet
Ph	phenyl
PLP	pyridoxal phosphate
nPr	propyl
iPr	isopropyl
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
Ts	p-toluenesulfonyl
UDP	uridine diphosphate

Introduction

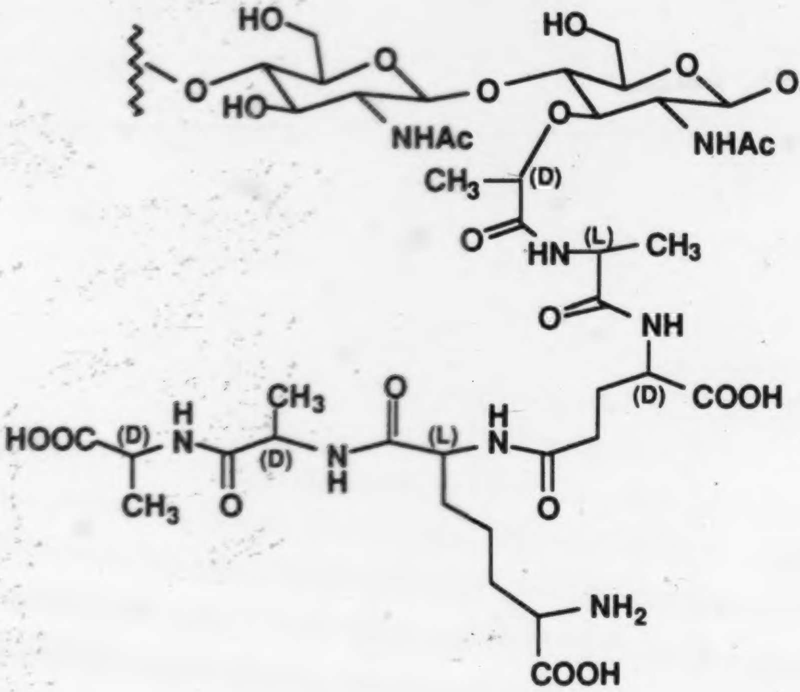
Diaminopimelic acid (DAP) is an important constituent used in the construction of bacterial cell wall peptidoglycan for virtually all Gram-negative bacteria and some gram-positive bacteria.[1-7] Peptidoglycan is a network structure whose integrity is essential to keep the bacterial cell alive under osmotic pressure.[8] This bio-polymer consists of repeating units of alternating β (1-4) linked N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) disaccharide, which is substituted through the D-lactyl group of N-acetylmuramic acid by L-Ala-γ-D-Glu-L-DAP-D-Ala tetrapeptide units (Figure 1). Peptide units on adjacent glycan chains are linked by means of bridges that extend from the C-terminal D-alanine of a peptide to the ω amino group of DAP residue of another peptide. This kind of cross-link is responsible for the rigidity of the peptidoglycan network thus formed (Figure 2). It has been shown that about 20% of the cross-links do not involve D-alanine but directly link the DAP residue of neighboring chains.[9]



DAP

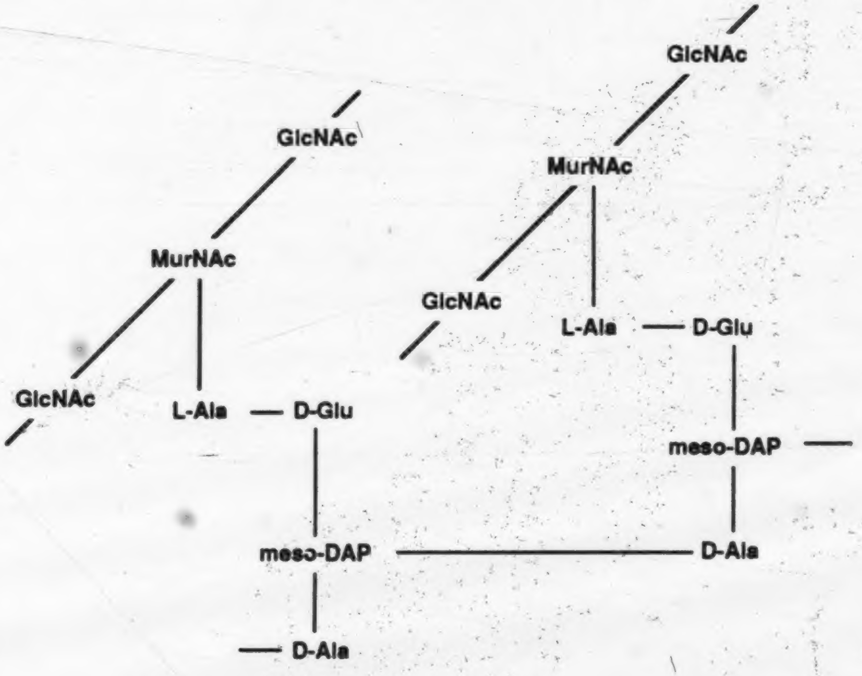
Peptidoglycan synthesis rests upon manufacture in the cytoplasm of the two nucleotides, UDP-GlcNAc and UDP-MurNAc-L-Ala-γ-D-Glu-L-DAP-D-Ala-D-Ala, which eventually form a peptidodisaccharide lipid phosphate. Incorporation of the lipid-transported disaccharide peptide units, as they emerge from the cytoplasmic membrane, into the pre-existing wall peptidoglycan is accomplished by transglycosylation and transpeptidation that require several membrane-bound enzymes.[10,11] Although the glycopentapeptide formation needs ATP as an energy source, transpeptidation does not require any input of energy but is made at the expense of a performed D-Ala-D-Ala peptide bond. Transpeptidase is able to utilize D-alanyl-D-alanine-terminated peptides as carbonyl donors to form transpeptide bonds with the amino group from DAP. Interference with the transpeptidation by β-lactam compounds gives them remarkable antibacterial properties.

Figure 1 Structure of Bacterial Peptidoglycan Monomer.



Besides its use in cross-linking adjacent peptide chains, DAP is also utilized to link lipoprotein by amide bond formation between the L-carboxy group of DAP and the ω amino group of lysine residue on the lipoprotein.[2,12] A molecule of lipoprotein is known to be attached at about every 10th peptidoglycan chain. Because of the significant roles played by DAP in the biosynthesis of bacterial wall peptidoglycan, rational drug design can be based on the inhibition of DAP production in bacteria. Since DAP is not found as a constituent of animal tissues and its biosynthetic pathway is absent in animals,[13,14] there is a reasonable expectation that an antibiotic acting on DAP biosynthesis is likely to be non-toxic to humans. Recent studies in several laboratories demonstrate that a number of compounds which inhibit the formation or metabolism of DAP by bacteria possess antibiotic activity.[15-21]

Figure 2 Crosslinking in Bacterial Peptidoglycan.



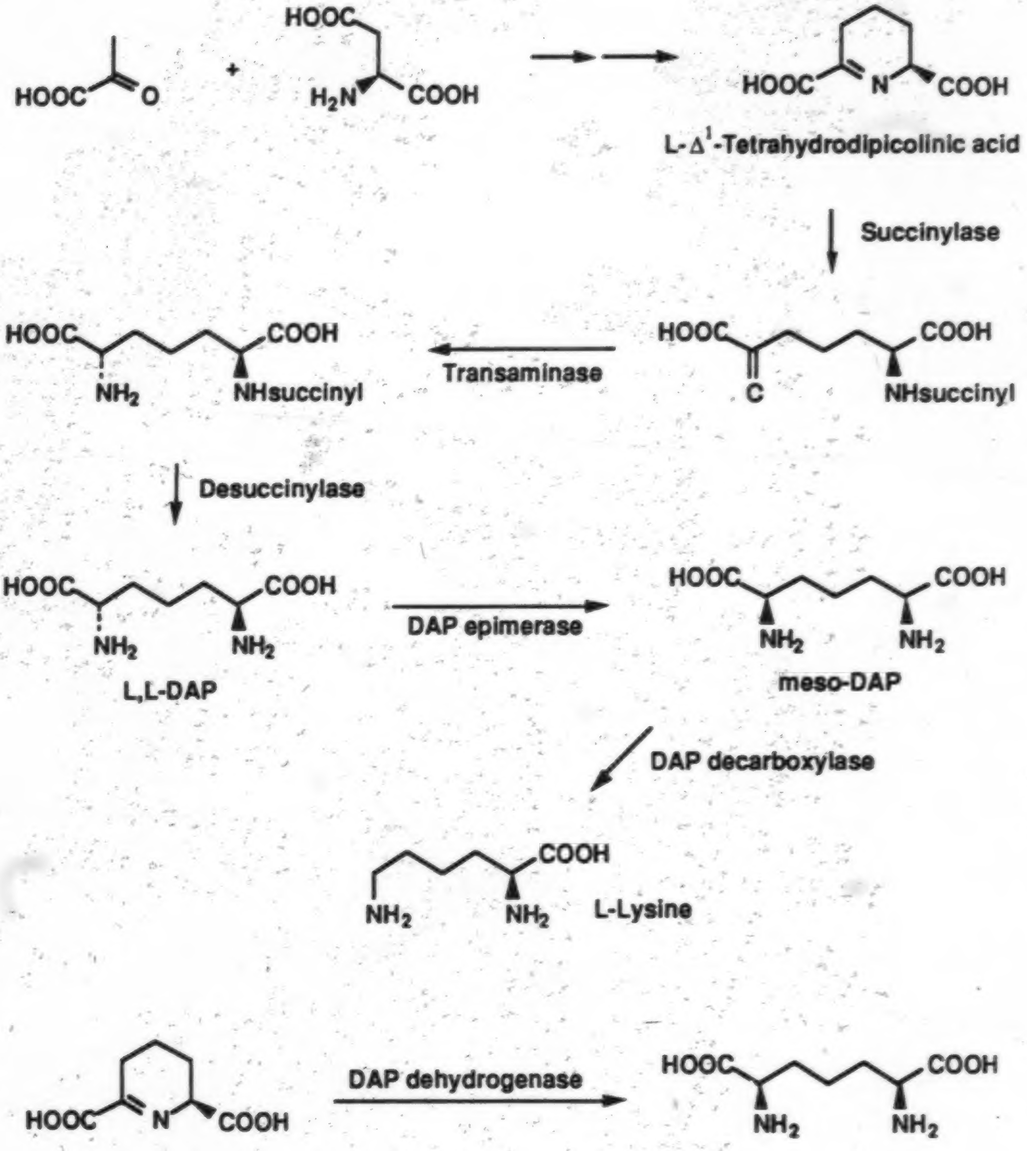
The biosynthetic pathway to DAP and lysine in bacteria has been extensively elucidated[22-30] (Figure 3). Tetrahydrodipicolinic acid, which is derived from pyruvate and aspartic acid in four steps by the action of four individual enzymes, is converted to 2-(succinylamino)-6-oxo-L-pimelic acid by DAP succinylase in the presence of succinyl-

CoA. The transamination of this α-keto acid is effected by an enzyme called DAP transaminase to afford N-succinyl L,L-DAP. After desuccinylation of the substrate under the catalysis of desuccinylase enzyme, the product L,L-DAP is epimerized to D,L-DAP (*meso*-DAP) by the action of DAP epimerase. The last step in this pathway is the decarboxylation of *meso*-DAP to lysine by DAP decarboxylase. An alternative pathway for DAP synthesis is also utilized by some bacteria, in which the tetrahydrodipicolinic acid is transformed directly to *meso*-DAP by reductive amination in the presence of NH_3 and NADPH, a process that is catalyzed by DAP dehydrogenase.[31,34]

Our efforts to discover new antibacterial agents aim at design and synthesis of inhibitors for key enzymes in the DAP biosynthetic pathway. Virtually every aspect of peptidoglycan biosynthesis in bacteria is a target on which the cell wall inhibitors can be investigated and developed.[35] Inhibition of the D-alanine synthesis by amino acid analogs[36-42] such as alafosfalin[43] and cycloserine[44] results in non-cross-linked peptide chains. Antibiotics with structure related to that of DAP are few in number,[45] and so far none has therapeutic utility. However the number of literature reports on the attempts to interdict the biosynthesis of DAP or interfere with its incorporation into peptidoglycan precursors has grown dramatically in the preceding five years.[15-21]

Although DAP epimerase was detected more than 30 years ago,[46] it was only recently purified from *Escherichia coli* and studied in detail by Wiseman and Nichols.[47] The enzyme is a monomer with molecular weight of 34,000. DAP epimerase is not a pyridoxal phosphate-dependent enzyme, based on the ultraviolet spectrum of the purified enzyme, and the lack of inactivation by carbonyl reagents such as hydroxylamine and

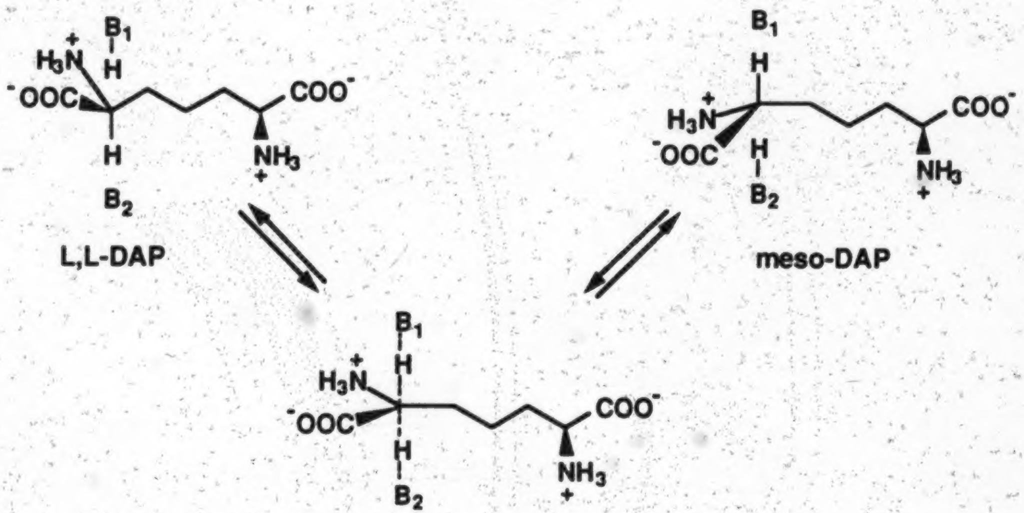
Figure 3 Diaminopimelate Pathway to L-Lysine in Bacteria.


HOOC
O
+
HOOC
H_2N
COOH
HOOC
N
COOH
L-Δ^1-Tetrahydrodipicolinic acid
Succinylase
HOOC
COOH
C
NHsuccinyl
Transaminase
HOOC
COOH
NH_2
NHsuccinyl
Desuccinylase
HOOC
COOH
NH_2
NH_2
L,L-DAP
DAP epimerase
HOOC
COOH
NH_2
NH_2
meso-DAP
DAP decarboxylase
COOH
NH_2
NH_2
L-Lysine
HOOC
N
COOH
DAP dehydrogenase
HOOC
COOH
NH_2
NH_2

Succinyl = $COCH_2CH_2COOH$

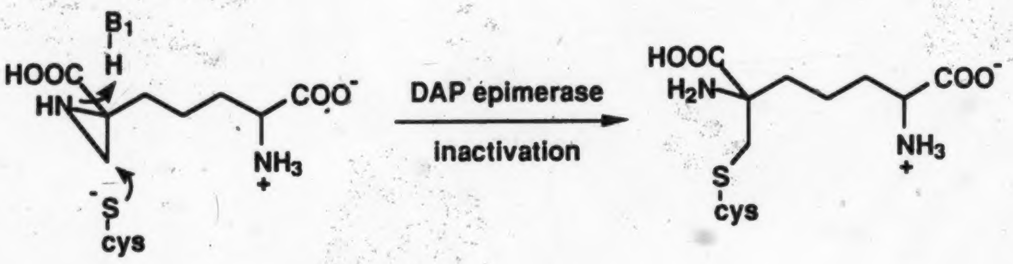
sodium borohydride. Kinetic analysis of an experiment in which the α-proton of the substrates exchange with tritium from solvent suggests a two-base mechanism for proton translocation. In this mechanism (Figure 4), one base abstracts the α-proton of the substrate from one side while a second base delivers a proton from the other side. It is possible that the formation of a high energy, unstabilized carbanion intermediate can be avoided by concerted proton transfers, which may result in a kinetic advantage. In these major aspects of the mechanism, DAP epimerase resembles proline racemase.[47] It was also suggested that one, but not both, of the proton acceptor sites was a thiol which reacts with iodoacetamide.

Figure 4 Mechanism Proposed for the Action of DAP Epimerase.



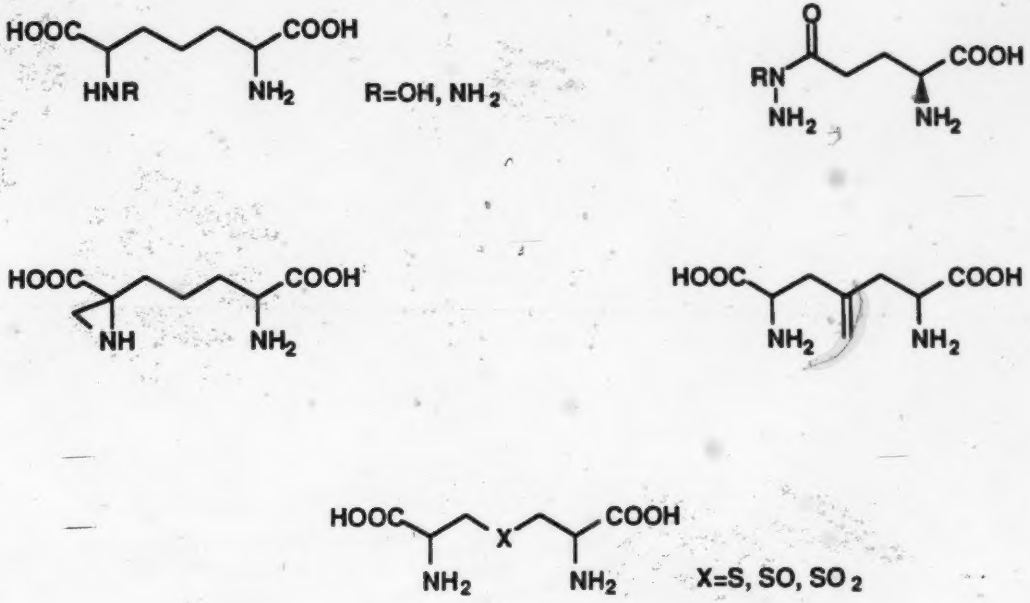
The *dap* gene encoding DAP epimerase has been isolated from a cosmid library.[48,49] The DNA sequence of the gene reveals an open reading frame of 822 bp; the deduced amino acid sequence of the DAP epimerase indicates a polypeptide of 274 amino acids with a calculated Mw of 30265, which is in agreement with the previous report of 34000 obtained from gel electrophoresis. By utilizing recombinant DNA techniques, Higgins and co-workers have been able to overproduce the DAP epimerase.[50] Recently, a DAP epimerase inactivator was synthesized[51] and used as a probe for study of the active site. Incubation of the enzyme with 2-(4-amino-4-carboxybutyl)-2-aziridine-carboxylic acid (AZIDAP) resulted in time-dependent irreversible inhibition (Figure 5). Tryptic digestion of the inactivated enzyme and peptide-mapping showed that AZIDAP was specifically and covalently bound to the enzyme at an unique peptide. Determination of the amino acid sequence of this peptide and comparison with the sequence deduced from the DNA sequence of the *dap* F gene indicated that cysteine 73 was labelled, thereby confirming its location in the active site.

Figure 5 Inactivation of DAP Epimerase by AZIDAP.



After the pure DAP epimerase became available, investigations were undertaken to design and synthesize enzyme inhibitors or inactivators for DAP epimerase.[19-21,51] More

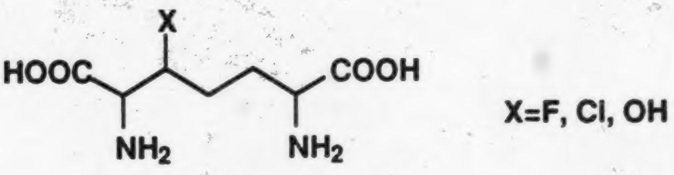
Figure 6 Compounds Tested as DAP Epimerase Inhibitors in Earlier Studies.


HOOC
COOH
HNR
NH2
R=OH, NH2
O
RN
NH2
COOH
NH2
HOOC
COOH
NH
NH2
HOOC
COOH
NH2
NH2
HOOC
X
COOH
NH2
NH2
X=S, SO, SO2

than a dozen modified DAP analogues have been synthesized as potential DAP epimerase inhibitors. The structures of some of these inhibitors are shown in Figure 6 and reflect the efforts aimed at developing synthetic compounds for the inhibition of DAP metabolism.

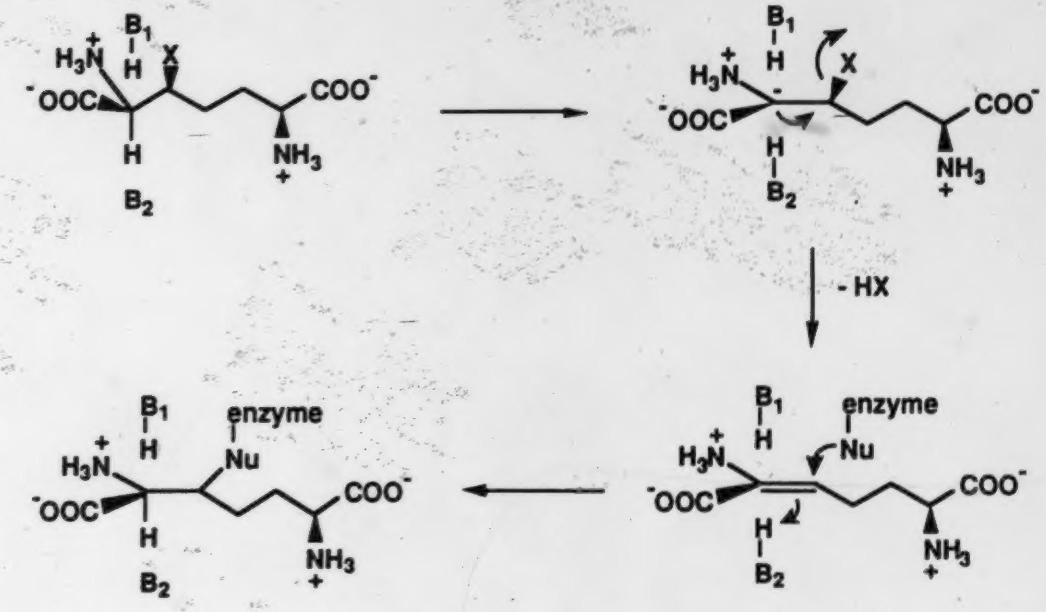
One of the goals of the present research was synthesis of DAP derivatives, in which a β-hydrogen is substituted by an electronegative atom or group (Figure 7). Inactivation

Figure 7 Target DAP Analogues.


X
HOOC
COOH
NH2
NH2
X=F, Cl, OH

of DAP epimerase by these substances may occur when the enzyme processes them in the same way as the natural substrate (DAP) (Figure 8) if anionic character develops at the α-position as a result of deprotonation. This incipient anion may prompt HX elimination to form a highly active enamine that can capture a nucleophilic residue of the enzyme, thereby binding covalently at the active site. Suicide inhibition[52,53] of alanine racemase occurs with β-fluoroalanine and β-chloroalanine.[54,55]

Figure 8 Possible Inactivation of DAP Epimerase by β-Substituted DAP.



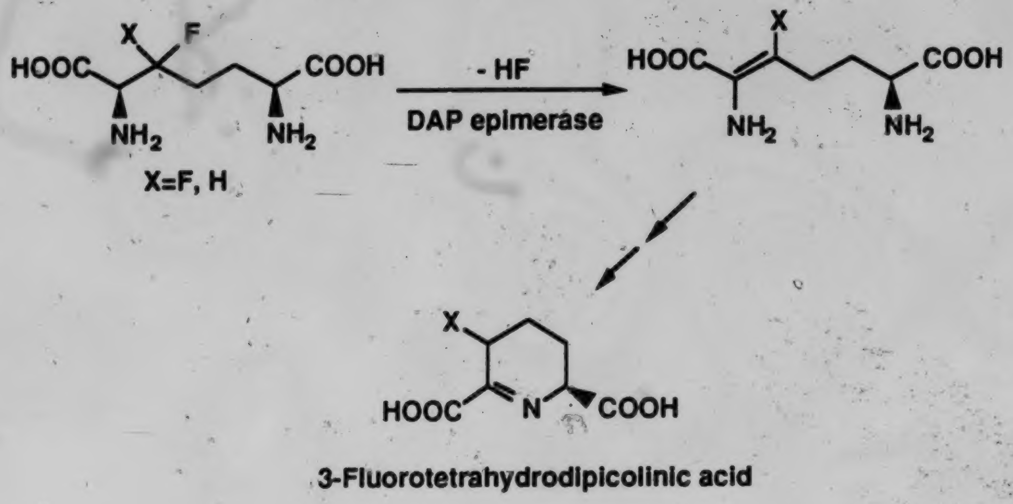
An important aspect of our synthetic approach is synthesis of all stereoisomers of the target DAP derivatives in stereochemically pure form. After our work was in progress, two publications have appeared that report the synthesis of β-chloro DAP and β-fluoro DAP as inhibitors for DAP epimerase.[20,21] However, these compounds were prepared as a mixture of all possible stereoisomers, by multi-step routes (8 steps for β-chloro DAP; 15

steps for β-fluoro DAP). Since enzyme inhibitors should bind tightly to the enzyme surface, the rotations around σ-bonds and translational motion are dramatically restrained when bound. Hence different stereoisomers could be expected to show quite different inhibitory activities.[62]

From the potential inhibitors shown in Figure 7, β-fluoro DAP was chosen as a primary synthetic goal, because fluorine has a small van der Waals radius (1.35 A) that is close to that of a hydrogen atom (1.20 A). In molecules where conformational recognition is important, minimal steric disturbance by a substituent is especially significant. Although the fluorine-carbon bond energy is relatively high, fluorine's ability to undergo β-elimination is well established.[52,58,59] Based on these arguments, β-fluoro DAP should resemble the natural substrate (DAP) more closely than the corresponding β-hydroxy and β-chloro analogs, and be capable of undergoing elimination which may lead to enzyme inactivation (Figure 8). Furthermore, visibility of fluorine nuclei by ^{19}F NMR spectrometry significantly facilitates the study of interactions with enzymes.

Other attractive targets for DAP-based enzymes are isomers of β,β-difluoro DAP (Figure 9, X=F). The presence of an additional fluorine at the β-position will make the α-proton more acidic. If β,β-difluoro DAP (Figure 9, X=F) undergoes epimerase-catalyzed HF elimination, the fluoro-enamine thus formed may undergo nucleophilic attack in an addition-elimination process. Alternatively, 3-fluorotetrahydrodipicolinic acid may be formed by the epimerase and could act as a potential inhibitor for DAP dehydrogenase (Figure 3). DAP dehydrogenase[34] is a NADPH dependent enzyme with a molecular weight of 80,000 that consists of two identical subunits. Studies show that this enzyme is highly specific for *meso*-DAP and operates on the D-center. Inhibition of the enzyme is expected to interfere with DAP production in bacteria that use the dehydrogenase to generate meso-DAP for their cell wall synthesis.

Figure 9 Hydrogen Fluoride Elimination of β-Fluoro and β,β-Difluoro DAP on DAP Epimerase.



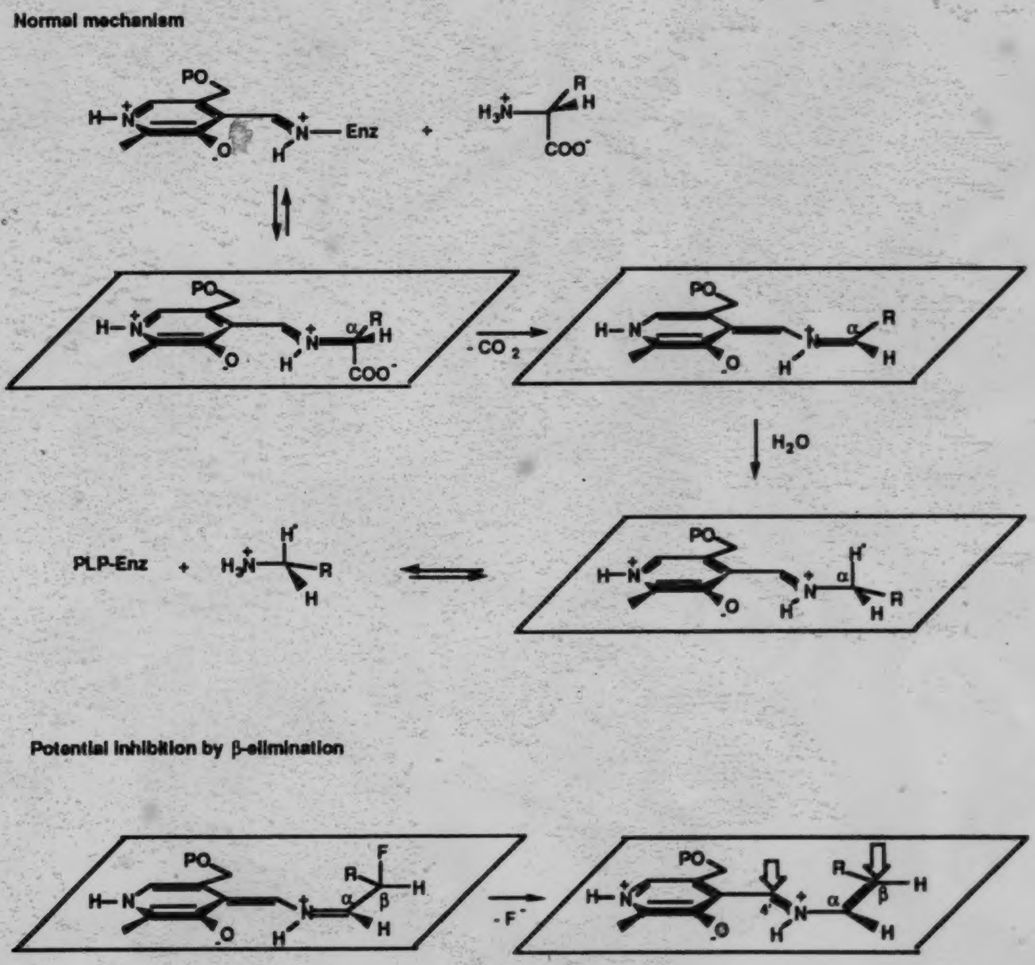
DAP decarboxylase is another potential target enzyme. This protein from both prokaryotic and eukaryotic sources is pyridoxal phosphate dependent and catalyzes the decarboxylation of meso-DAP exclusively at the D-center with an unusual inversion of configuration to give L-lysine.[64,65] The enzyme is highly specific for the meso-isomer, and the D,D- and L,L-isomers of DAP are neither substrates nor effective inhibitors.[66] Inhibition of the decarboxylase by L-lysine is well-documented[66-71] and may assist regulation of lysine biosynthesis.[70] A variety of structurally related compounds have been examined as inhibitors for the decarboxylase. Among them are α-difluoromethyl DAP,[17] α-aminopimelic acid,[66,69] and unsaturated analogues of DAP,[18] in addition to the sulfur-containing analogues, N-hydroxy and N-amino DAP derivatives[17] shown in Figure 6.

The general mechanism for a pyridoxal phosphate dependent decarboxylase is depicted in Figure 10. Based on stereoelectronic considerations, during decarboxylation of α-amino acids the bond between the α-carbon and the carbonyl carbon of the substrate is expected to be nearly perpendicular to the plane of the cofactor's conjugated π system.[72-74]

The cofactor essentially stores the electrons of the cleaved bond until protonation from solvent can occur.

If a substrate analog is used which has a leaving group (e.g. fluorine) attached to a β-carbon of the α-amino acid, the intermediate (shown in Figure 10) may cause fluoride elimination to generate an electron-deficient conjugated system. Again stereoelectronic effects for the elimination require that the bond between the β-carbon and its attached

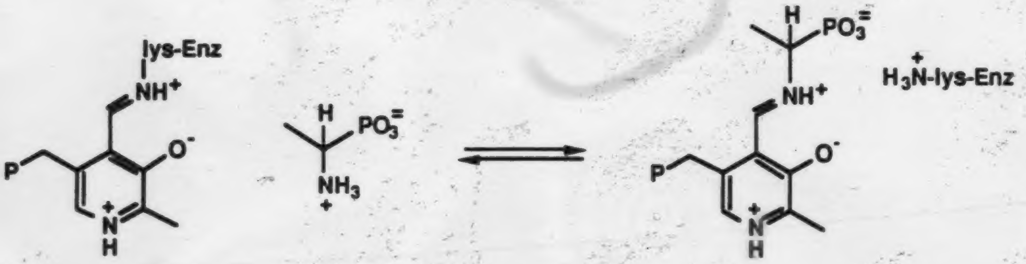
Figure 10 Mechanism of DAP Decarboxylase and Possible Mode of Inhibition by β-Fluoro DAP.


Normal mechanism
PO
H–N
N–Enz
O
H
+
H_3N
R
H
COO⁻
α
- CO 2
H_2O
PLP-Enz
H'
Potential inhibition by β-elimination
F
β
- F⁻
4'

leaving group be aligned nearly perpendicular to the plane of the conjugated cofactor-inhibitor complex. The elimination produces a highly reactive intermediate (shown in Figure 10) which is prone to attack by nucleophiles (as indicated by the hollow arrows) at the β-carbon or at C-4' of the cofactor.[75,76] This may lead to covalent attachment of the cofactor-inhibitor complex to a group in the enzyme active site, or alternatively, it may cause cleavage at C-4' to release a reactive enamine which can be attacked by the enzyme-cofactor complex in an electrophilic fashion.[75,77-79]

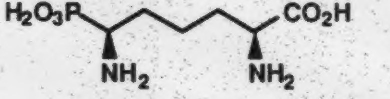
A second class of potential inhibitors that were targeted are α-aminophosphonic acids. These compounds have a phosphonic acid group in place of a carboxylic acid functionality and are close mimics of α-amino acids. Phosphorus-modified analogues of amino acids and peptides have been widely utilized to achieve a variety of bioactivities,[80-88] especially as transition-state enzyme inhibitors.[56,82-84] The analogue of alanine, (1-aminoethyl)phosphonic acid (Ala-P), was shown[89-92] to be potent inhibitor for alanine racemase from Gram-positive bacteria. The racemase is a pyridoxal phosphate containing enzyme that catalyzes the racemization of L- and D-alanine. The inactivation of the racemase by the aminophosphonic acid (Ala-P) occurs via a time-dependent, slow-binding

Figure 11 Mechanism of Inactivation of Alanine Racemase by Ala-P.

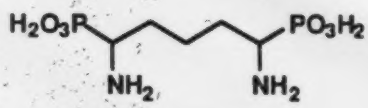


process[93] in which Ala-P initially binds in a weak, reversible fashion. This is followed by a conformational change to yield an enzyme-Ala-P complex that dissociates with a half-life of 25 days. This conformational change may also be responsible for the inaccessibility of the active site Schiff base to sodium borohydride reduction. The enzyme-Ala-P complex was subsequently determined[94] to be a protonated Ala-P-PLP aldimine (Figure 11) by solid-state ^{15}N NMR techniques. Phosphinic acid analogues which contain only a monophosphate anion at pH's where phosphonate is doubly ionized, do not lead to time-dependent, slow-binding inactivation.[89] The related β-chloro Ala-P and β-fluoro Ala-P were also shown to be strong inhibitors of alanine racemase and of D-alanine-D-alanine ligase.[95,96]

On this basis, the phospho analogues of DAP shown below could inactivate the pyridoxal phosphate dependent DAP decarboxylase in a similar fashion. Furthermore, these DAP phosphonates could be potential inhibitors for DAP epimerase. The structural similarity between DAP and its phosphonate analogue is likely to allow binding to the enzyme. Compared with DAP, the phosphorus derivative carries an additional negative charge, which may influence the normal protonation or deprotonation of the two bases in the enzyme active site that serve a vital function for the epimerase.



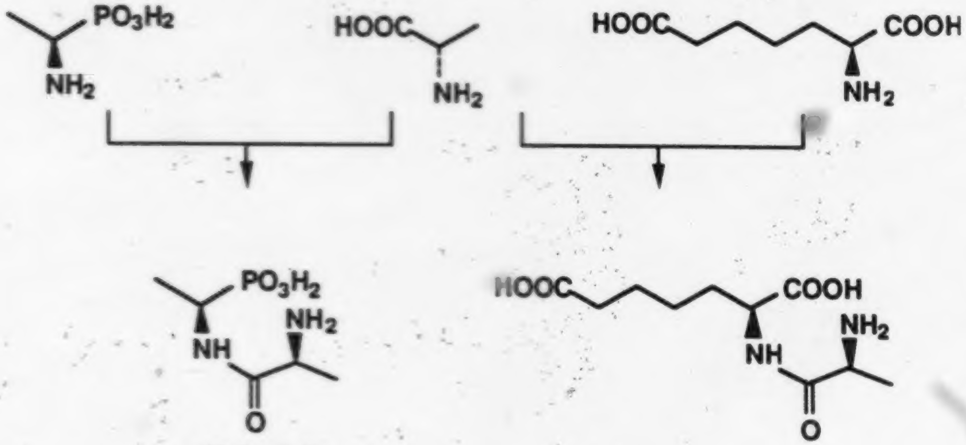
DAP monophosphonate analogue



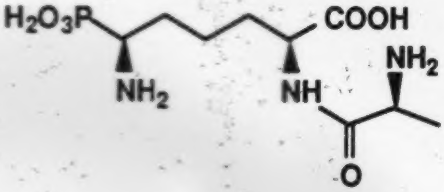
DAP diphosphonate analogue

In many cases, compounds that inhibit individual enzyme(s) responsible for cell wall peptidoglycan synthesis *in vitro* do not show antibacterial activity *in vivo*.[15,17-19,97] Lack of the correlation between enzyme inhibition and bacterial growth inhibition is often attributable to a poor cross-membrane transport of the inhibitor into cell cytoplasm where the target enzyme is located. However, it is well-established that bacteria utilize peptides as both carbon and nitrogen sources for their growth.[98,99] The needed peptides are transported into cells through an unique peptide transport system (peptide permeases).[100,101] By taking advantage of this system a drug molecule can enter the cell to attack the intracellular enzymes. This so-called 'warhead-delivery' strategy[102-106] involves incorporation of the drug molecule into a suitable peptide that is then transported through the peptide permease into the cell, where the drug molecule is released by virtue of intracellular hydrolysis of the peptide. Alafosfalin serves as a remarkable example for the strategy. Alafosfalin[107] is a dipeptide of L-alanine and 1-aminoethylphosphonate (Figure 12). ^{14}C-Incorporation experiments indicate that the L-Ala-L-Ala-P uses active energy-linked transport by an L,L-stereospecific peptide permease, for which L,L-alanylalanine can compete with alafosfalin. After being transported into the cell, Ala-P is freed from alafosfalin by peptidase cleavage. It then inhibits alanine racemase in the cytoplasm by the mechanism discussed before (Figure 11) to express its antibacterial activity. In another example, Gilvarg and colleagues[15] observed that 2-aminopimelic acid (Figure 12) is an inhibitor of DAP succinylase (Figure 3) but is devoid of antibacterial activity. However, incorporation of the compound into a di- or tripeptide yields derivatives that are antibiotics against a range of Gram-negative organisms.

Figure 12 Antibacterial Peptides from Non-antibacterial Precursors.


Alafosfalin

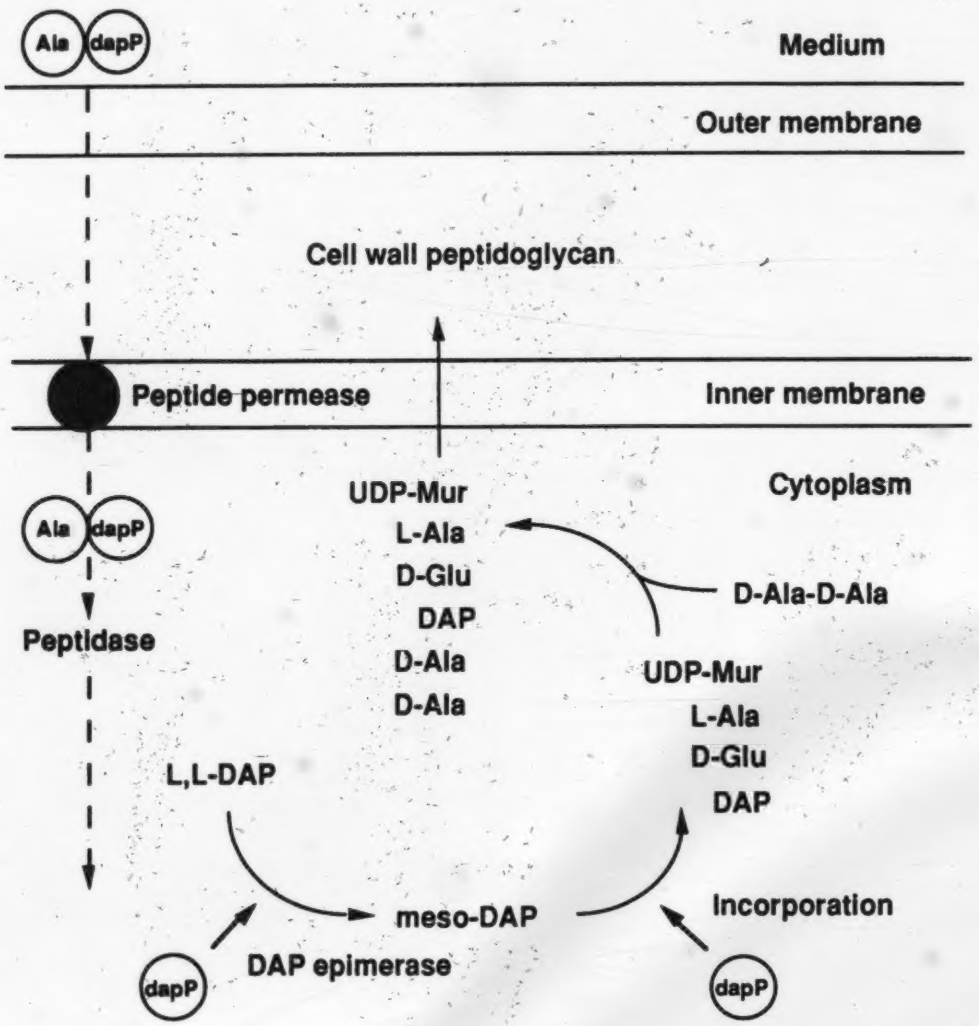
To increase the possibility that the phosphonate analogues of DAP are transported into bacterial cells, thus enhancing their antibacterial activity, the present work also aimed at synthesis of dipeptides with alanine (see example below).



In addition to attacking DAP enzymes, the DAP phosphonate analogues may interfere with DAP incorporation into the peptidoglycan precursor (Figure 13). Alternatively, if DAP phosphonate is incorporated into the disaccharide peptide chain in place of DAP, the subsequent attachment of D-alanyl-D-alanine may be aborted because of the phosphonate modification. The replacement of DAP with its phosphonate analogue in the disaccharide pentapeptide chain may also abort the vital cross-peptidation between DAP and D-alanine.

Incorporations of other DAP analogues into bacterial peptidoglycan have been reported.[108-110]

Figure 13 Possible Mechanism of Action of L-Ala-DAP Phosphonate in Gram-negative Bacteria.

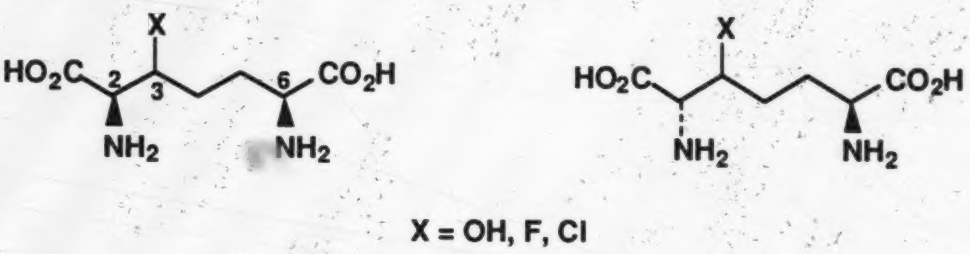

Ala
dapP
Medium
Outer membrane
Cell wall peptidoglycan
Peptide permease
Inner membrane
Cytoplasm
Ala
dapP
Peptidase
UDP-Mur
L-Ala
D-Glu
DAP
D-Ala
D-Ala
D-Ala-D-Ala
UDP-Mur
L-Ala
D-Glu
DAP
L,L-DAP
meso-DAP
DAP epimerase
Incorporation
dapP
dapP

Results and Discussion

Synthesis of β-Hydroxy and β-Fluoro Diaminopimelic Acid Isomers

In designing rational mechanism-based inhibitors or inactivators for diaminopimelic acid-metabolising enzymes (e.g. DAP epimerase and DAP decarboxylase), the compounds shown in Figure 14 in which the hydrogen at C-3 position is substituted by a potential leaving group (hydroxy, fluorine or chlorine atom), were chosen as the target molecules.

Figure 14 Target β-Substituted DAP Analogues.



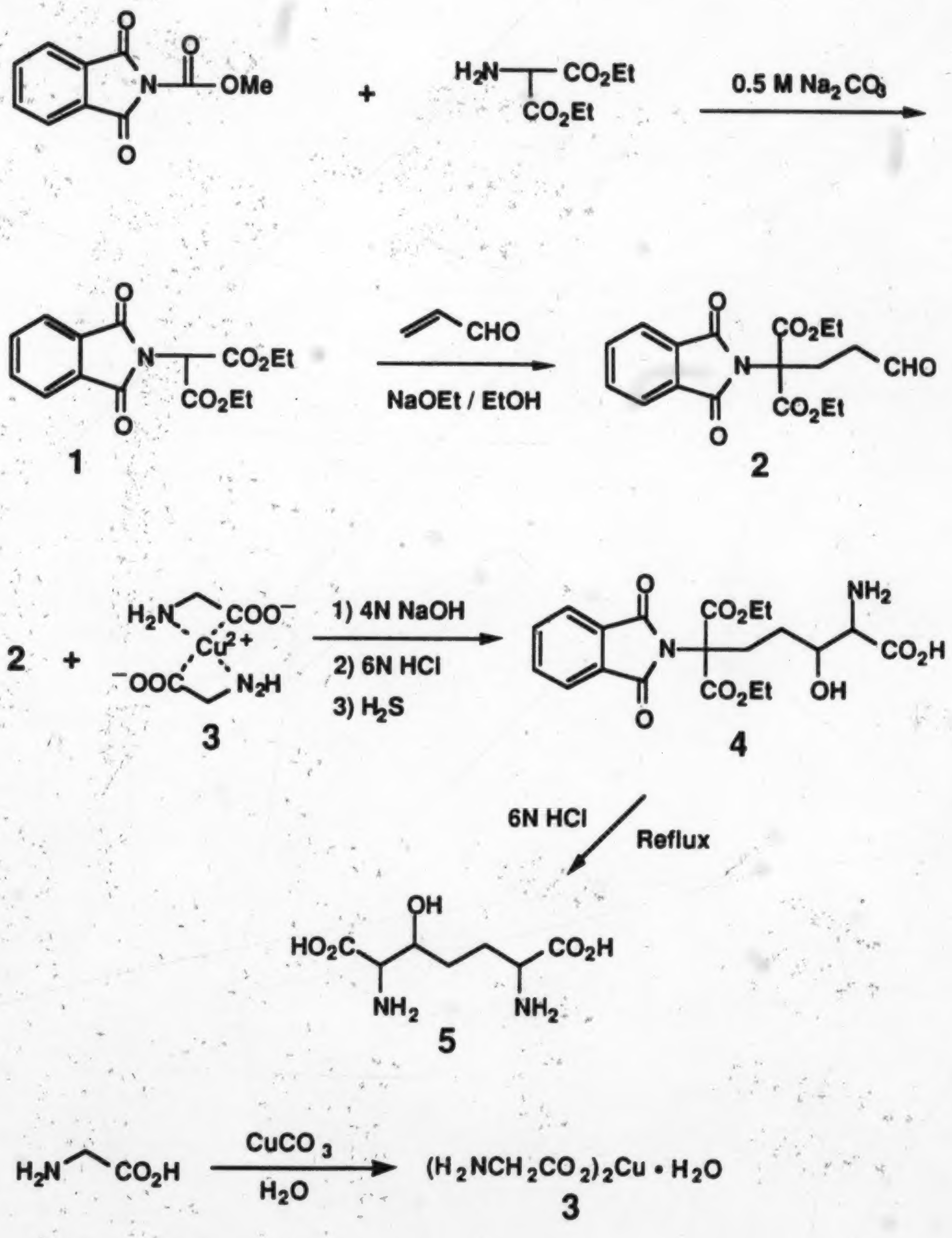
The synthesis of β-hydroxy DAP was the first goal because in addition to being a potential enzyme inhibitor, it or its protected derivatives are ideal intermediates for synthesis of β-fluoro DAP or β-chloro DAP analogues. Dehydroxyfluorination of β-hydroxy α-amino acid derivatives has been well developed during the last decade.[111-115,155]

In 1966, Sundharadas and Gilvarg[108] reported a synthesis of β-hydroxy DAP as a mixture of all eight possible stereoisomers. Four racemic pairs of these isomers were separated by a tedious fractional recrystallization that employed four different aromatic sulfonic acids to form salts. These racemic pairs have different effects on the growth of a DAP-requiring auxotroph of *E.coli* in a synthetic medium totally devoid of DAP. Although this synthetic method could not meet our requirement to make these isomers in stereochemically pure form, it was still desirable to prepare reference samples that could be tested in dehydroxyfluorination procedures. The modified literature method[108] was therefore used to synthesize the stereoisomeric mixture of compounds **5**.

Diethyl phthalimidomalonate (**1**) (Scheme 1), available from reaction of diethyl aminomalonate with N-methoxycarbonyl phthalimide in 58% yield, underwent Michael addition to acrolein in the presence of sodium ethoxide to give γ-phthalimido-γ, γ-dicarboethoxy butyraldehyde (**2**) in 43% yield. Copper glycinate (**3**)[116] was made by heating 2 equivalent of glycine with one equivalent copper carbonate in water. This underwent aldol condensation with **2** in 4N NaOH at 5 °C. After acidification with 6N HCl and removal of copper with hydrogen sulfide, the reaction mixture was heated to reflux in 6N HCl for 10 h to remove the phthalyl protecting group, hydrolyze the two ester groups, and decarboxylate to afford **5** in 19% yield.

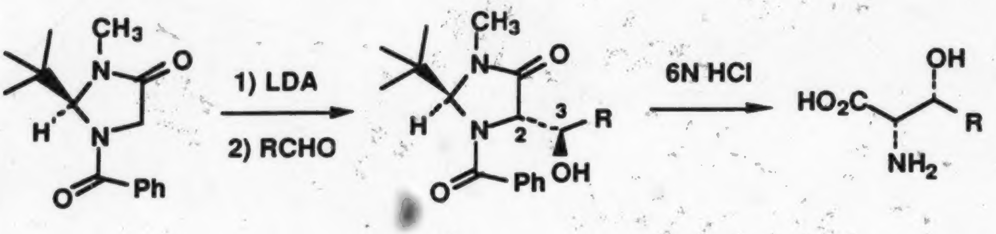
With the β-hydroxy DAP isomer mixture available, highly stereoselective methods to make its pure stereoisomers were investigated. During the last decade, a large number of synthetic methods have been established to stereoselectively synthesize β-hydroxy amino acids.[118-130] Among them, the one introduced by Seebach appeared most attractive. In this method[121] (Figure 15), imidazolidinone chiral auxiliary compounds are employed to serve as glycine-equivalents that, upon enolization by lithium diisopropyl amide (LDA), are able to undergo condensations with a variety of aldehydes to yield corresponding aldol adducts. These readily hydrolyze in aqueous HCl to β-hydroxy amino acids. The high stereoselectivity of this aldol condensation can be rationalized as follows. When the electrophilic carbonyl compound approaches the chiral enolate, the entry *cis* to the bulky tert-butyl group is sterically encumbered; therefore the attack of an electrophile *anti* to the

Scheme 1


O
O
OMe
N
O
+
H_2N
CO_2Et
CO_2Et
0.5 M Na_2CO_3
N
CO_2Et
CO_2Et
1
CHO
NaOEt / EtOH
CO_2Et
CHO
CO_2Et
2
2
+
H_2N
COO^-
Cu^{2+}
^-OOC
N_2H
3
1) 4N NaOH
2) 6N HCl
3) H_2S
CO_2Et
NH_2
CO_2H
CO_2Et
OH
4
6N HCl
Reflux
OH
HO_2C
CO_2H
NH_2
NH_2
5
H_2N
CO_2H
$CuCO_3$
H_2O
$(H_2NCH_2CO_2)_2Cu \cdot H_2O$
3

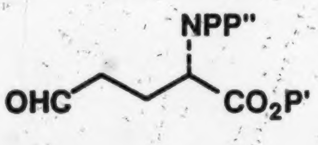
tert-butyl group proceeds predominantly to generate an *anti* diastereomer. Another significant advantage of this process, is control of the stereochemistry at the C-3 hydroxy center (d.e.>95%). This may be due to a delicate conformational interaction between the chiral enolate and the trigonal carbonyl group of the aldehyde during the condensation as proposed by Seebach and co-workers.[121]

Figure 15 Synthesis of β-Hydroxy Amino Acids by Seebach's Condensation.



The structure of β-hydroxy DAP (Figure 14) suggests that the target molecule could be constructed by an aldol reaction between the Seebach imidazolidinone and a five-carbon aldehyde. This requires that the aldehyde possess a fully protected amino acid group at one end as illustrated in Figure 16. Naturally-occurring L-glutamic acid seemed to be an ideal

Figure 16 Structure of the Target Aldehyde.

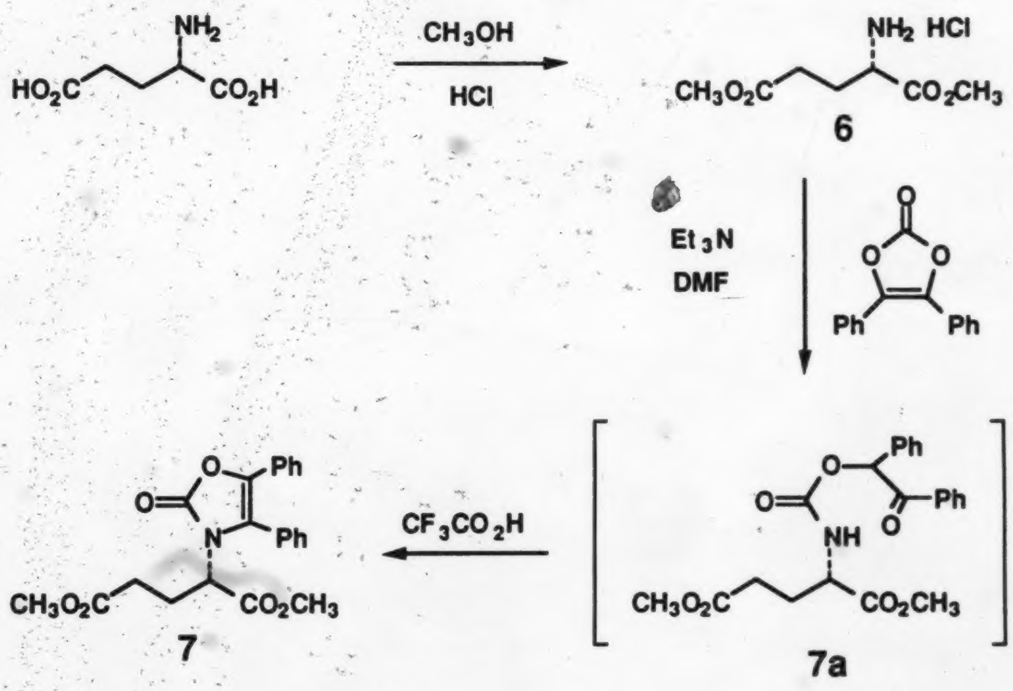


P, P', P" = protecting groups

starting material because it is very inexpensive. Moreover, the readily available D-glutamic acid would provide access to the opposite enantiomer of the aldehyde, thereby allowing control of stereochemistry at the distal end (C-6) of β-hydroxy DAP.

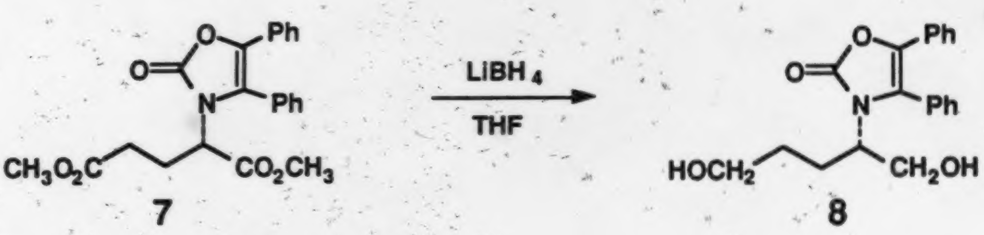
L-Glutamic acid was easily converted to its dimethyl ester **6** in 93% yield by stirring in dry methanol that had been saturated with anhydrous HCl gas. The commercially available 4,5-diphenyl 1,3-dioxol-2-one was known to be able to fully protect an amino group by forming an oxazolone[131] (Scheme 2). Treatment of **6** with this reagent under basic conditions gave the acyclic intermediate **7a**, which underwent cyclization in the presence of trifluoroacetic acid to afford **7** in 11% yield.

Scheme 2


NH2
HO2C
CO2H
CH3OH
HCl
NH2 HCl
CH3O2C
CO2CH3
6
Et3N
DMF
O
O
O
Ph
Ph
Ph
O
O
Ph
NH
O
CH3O2C
CO2CH3
7a
CF3CO2H
O
Ph
O
N
Ph
CH3O2C
CO2CH3
7

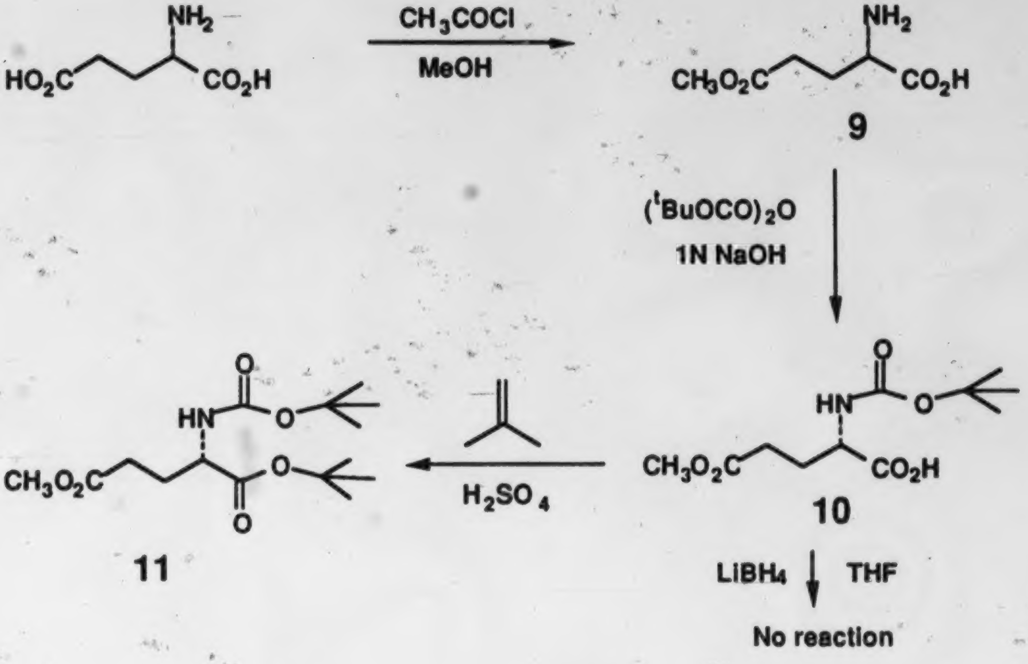
The next goal was to selectively reduce the less hindered methyl ester group to the corresponding alcohol without affecting the other ester at the sterically shielded amino end (Scheme 3). However, when the dimethyl ester **7** was treated with one equivalent of lithium borohydride, the dihydroxy oxazolone **8** (38% yield) resulting from non-selective reduction, and the starting material **7** appeared in equal amounts, along with small quantities of two mono alcohols.

Scheme 3



In an alternative approach, L-glutamic acid was selectively esterified following a literature procedure[132] to yield its γ-methyl ester **9** in 40% yield (Scheme 4). The amino group in the mono ester **9** was subsequently protected with a tert-butoxycarbonyl (Boc) group by di-tert-butyl pyrocarbonate. Unfortunately, this transformation proceeded in very low yield (18%). Selective reductions of ester functionalities in the presence of carboxylic acid by special reagents are well precedented.[133] However, attempts to reduce the methyl ester **10** failed with lithium borohydride in THF. To overcome this problem, the free carboxylic acid group in **10** was protected as an ester chosen to be more stable to reduction than the existing methyl ester. The N-Boc-L-glutamic γ-methyl ester **(10)** was esterified with isobutylene using concentrated sulfuric acid as catalyst. Unfortunately, the product **11** was obtained in a very low yield (12%). The two consecutive low-yielding transformations made this approach impractical. To overcome these problems, N-carbobenzyloxy-L-

Scheme 4

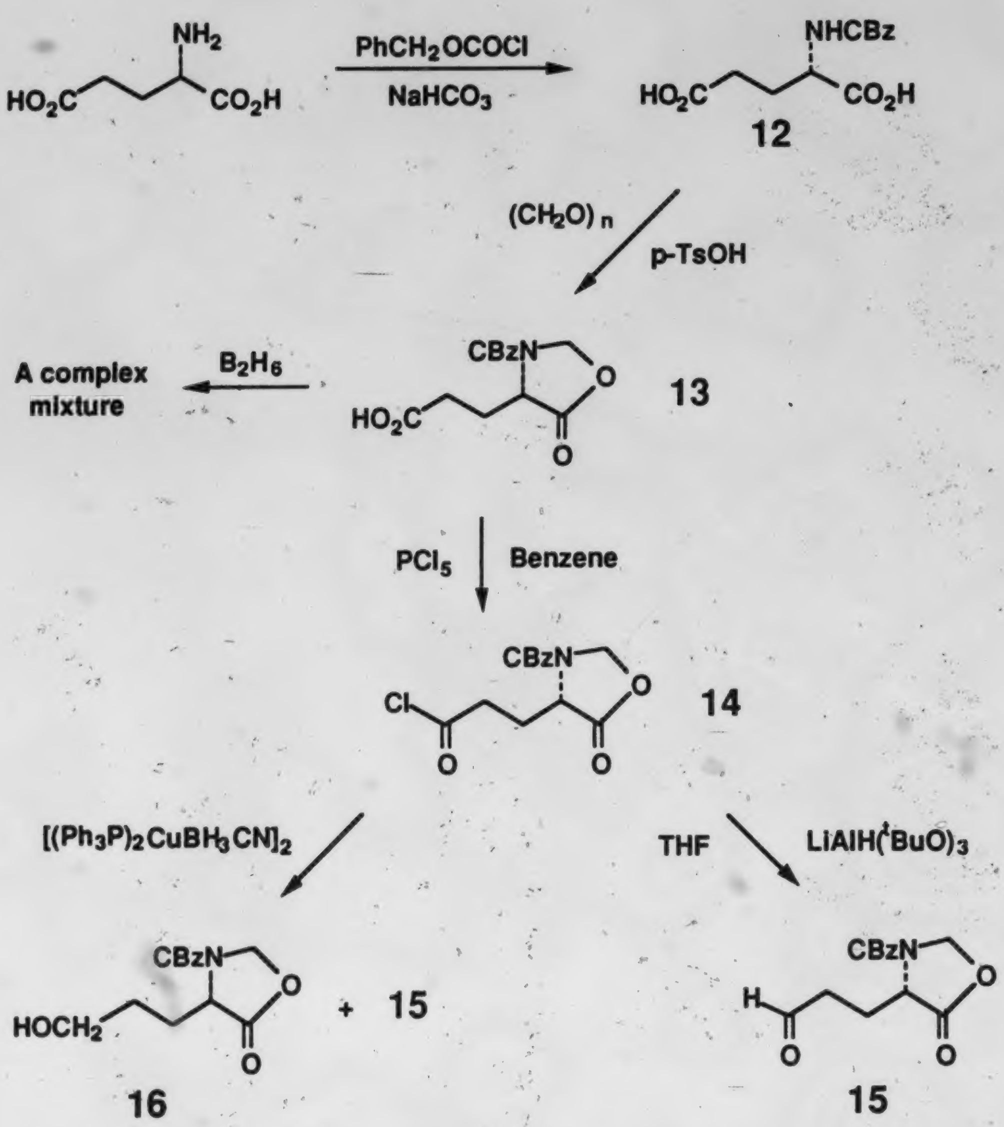


NH$_2$
HO$_2$C
CO$_2$H
CH$_3$COCl
MeOH
NH$_2$
CH$_3$O$_2$C
CO$_2$H
9
(^{t}BuOCO)$_2$O
1N NaOH
O
HN
O
CH$_3$O$_2$C
CO$_2$H
10
LiBH$_4$
THF
No reaction
H$_2$SO$_4$
O
HN
O
CH$_3$O$_2$C
O
O
11

glutamic acid (**12**), readily available from reaction of L-glutamic acid with carbobenzyloxyl chloride, was heated to reflux with paraformaldehyde in the presence of catalytic amount of p-toluenesulfonic acid to give oxazolidinone **13** (Scheme 5).[226] The amino acid end is masked in **13** and the free carboxylic acid end is available for functionalization to the desired aldehyde. Although diborane is known to be capable of converting carboxylic acids to corresponding alcohols,[134,135] reaction of **13** with diborane, generated from sodium borohydride and boron trifluoride etherate produces a complex mixture.

Acid **13** could be converted to the corresponding acyl chloride **14** (87% yield) by treatment with phosphorus pentachloride in benzene.[227] Attempted reduction of **14** to the

Scheme 5


PhCH$_2$OCOCl
NaHCO$_3$
12
(CH$_2$O)$_n$
p-TsOH
A complex mixture
B$_2$H$_6$
13
PCl$_5$
Benzene
14
[(Ph$_3$P)$_2$CuBH$_3$CN]$_2$
THF
LiAlH(^{t}BuO)$_3$
16
+ 15
15

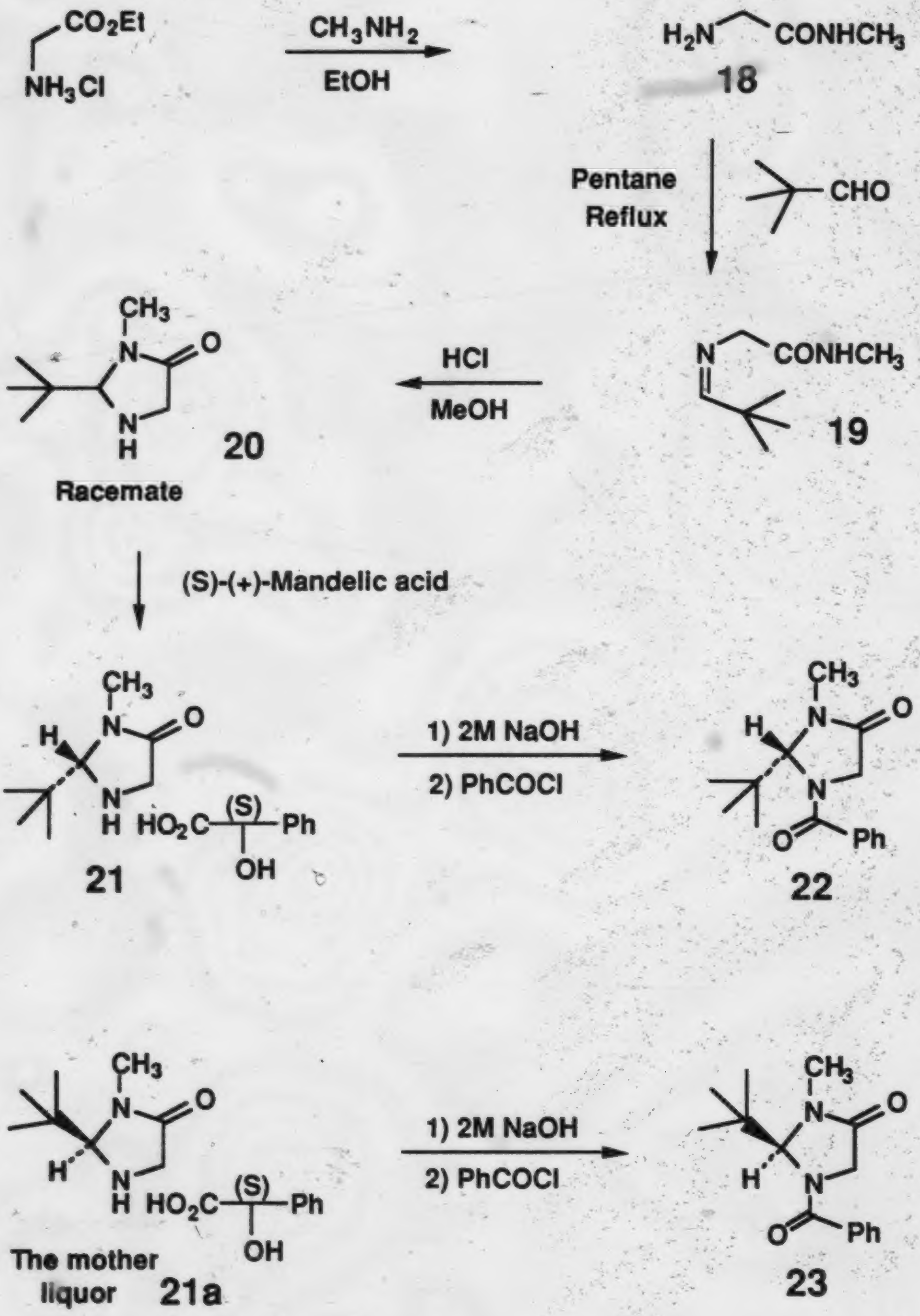
$$\mathrm{CuSO_4\cdot5H_2O} + \mathrm{Ph_3P} + \mathrm{NaBH_3CN} \longrightarrow \underset{\textbf{17}}{[(\mathrm{Ph_3P})_2\mathrm{CuBH_3CN}]_2}$$

CBz = PhCH$_2$OOC-corresponding alcohol **16** with sodium borohydride resulted in the destruction of the oxazolidinone ring. There are a number of other methods available for reduction of acyl chlorides to aldehydes.[136-144] In another approach,[144] the acyl chloride **14** was treated with bis(triphenylphosphine)copper cyanoborohydride dimer (a mild reagent made from copper sulfate hydrate, triphenylphosphine, and sodium cyanoborohydride) to give a mixture of the desired aldehyde **15** (7%) and the alcohol **16** (15%). However, use of lithium tri-*tert*-butyloxyaluminum hydride at -78°C in dry THF gives the aldehyde **15** in 80% yield.[145] This somewhat unstable material could be used directly in the next transformation without further purification.

With aldehyde **15** in hand, the next step was to make the Seebach chiral auxiliary compounds. Literature procedures[146,147] were followed to synthesize these materials. Ethyl glycinate hydrochloride reacts with monomethyl amine in absolute ethanol to give N-methyl amide **18** in quantitative yield, which is then transformed to imine **19** (76% yield) with pivalaldehyde upon heating in pentane with azeotropic removal of water. The ring closure occurs in anhydrous methanol saturated with hydrogen chloride gas to afford cyclic imidazolidinone **20** as a racemic mixture. The racemates were resolved by fractional recrystallization of salts of (S)-(+)-mandelic acid. The crystals containing the (S) isomer of the imidazolidinone as its mandelic acid salt **21** were neutralized with sodium hydroxide, the free base was acylated with benzoyl chloride to yield (R) isomer **22** in 89% yield (N-benzoylation changes the assignment at the chiral center).

The mother liquor containing the (R) isomer of the imidazolidinone salt was treated similarly to afford the enantiomer **23** (45% yield). The optical rotations of the two enantiomers **22** ([α] -116, c 1, CH_2Cl_2) and **23** ([α] +124, c 1, CH_2Cl_2) were in good agreement with literature values.[147]

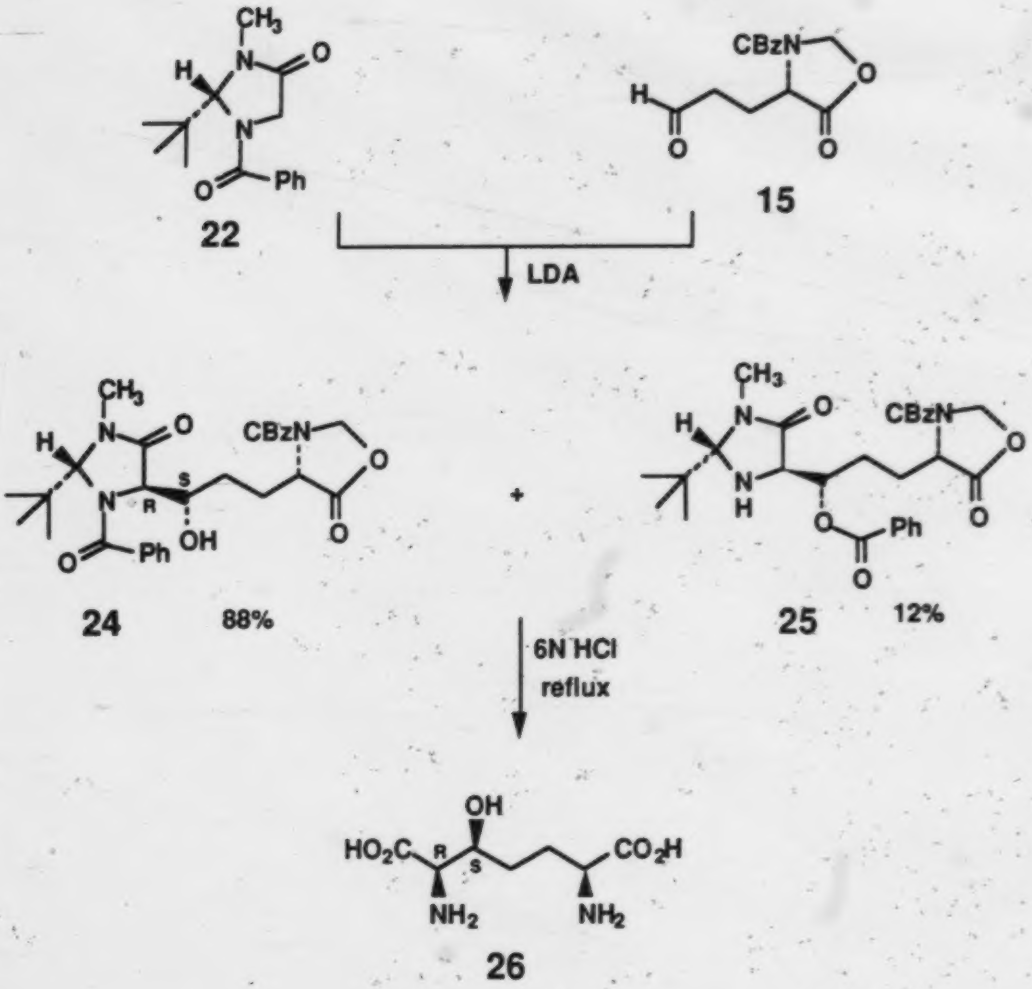
Scheme 6


CO2Et
NH3Cl
CH3NH2
EtOH
H2N CONHCH3
18
Pentane
Reflux
CHO
N CONHCH3
19
HCl
MeOH
CH3
N
O
N
H
20
Racemate
(S)-(+)-Mandelic acid
H
HO2C (S) Ph
OH
21
1) 2M NaOH
2) PhCOCl
Ph
22
The mother
liquor
21a
23

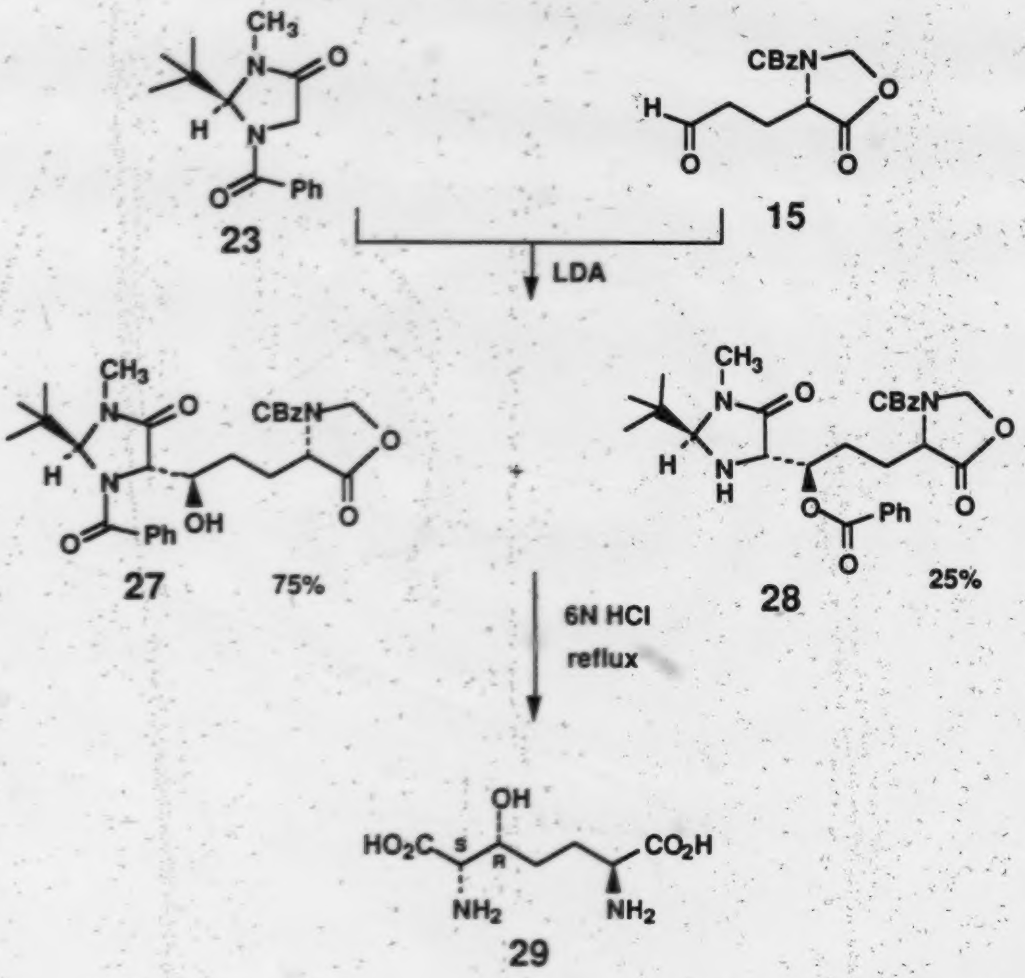
The enolate of **22**, generated with LDA, reacts with aldehyde **15** to produce a mixture of aldol adduct **24** and rearranged compound **25** in a ratio of 88 : 12 (Scheme 7). The ratio was found to change to 25 : 75 when the reaction was quenched with 1N HOAc instead of half-saturated NH_4Cl solution. This rearrangement to produce **25** involves migration of the benzoyl group from nitrogen to oxygen, and was also observed by Seebach and co-workers.[121] The yield for the mixture of **24** and **25** after chromatographic purification was 49%. Attempted separation of them by flash chromatography and preparative TLC resulted in the decomposition. However, since the benzoyl group migration does not alter the stereochemistry of the hydroxyl, the mixture of **24** and **25** was hydrolyzed in 6N HCl at reflux for 8h to produce (2R,3S,6S) β-hydroxy DAP (**26**) in 99% yield. Its stereochemistry was assigned by comparison with analogous compounds reported in the literature.[121] Only one stereoisomer was observed in the ^1H NMR and ^{13}C NMR spectra; each stereoisomer of β-hydroxy DAP can be distinguished by NMR spectroscopy as discussed below.

The aldol condensation between aldehyde **15** and enantiomeric imidazolidinone **23** proceeded similarly to give a mixture of the aldol adduct **27** and the migration product **28** in a ratio of 75 : 25 in 50% yield (Scheme 8). Hydrolysis of the mixture as before gives the (2S,3R,6S) β-hydroxy DAP **29** in 99% yield. Again the stereochemical purity of the isomer was confirmed by its ^1H NMR and ^{13}C NMR spectra in which only one isomer was observed. As expected, The ^1H NMR spectrum of a mixture of isomers **26** and **29** in a ratio of 1 : 1.6 showed differences in chemical shifts for the two isomers; the doublet due to the C-2 proton of **29** was more upfield than the corresponding one due to **26**. The ^{13}C NMR spectrum of a mixture of **26** and **29** also displays differences in chemical shifts.

Scheme 7


22
15
LDA
24
88%
+
25
12%
6N HCl
reflux
26
CH3
N
O
H
CBzN
Ph
OH
R
S
HO2C
CO2H
NH2

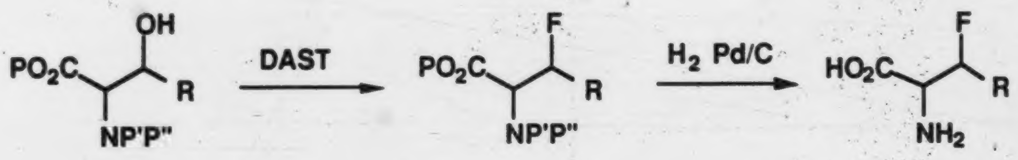
Scheme 8


CH3
O
H
N
Ph
23
CBzN
H
O
15
LDA
27
OH
75%
28
25%
6N HCl
reflux
HO2C
s
R
CO2H
NH2
29

The Seebach aldol condensation allows control of the stereochemistry at the C-2 center and C-3 of β-hydroxy DAP through use of the (S) or (R) imidazolidinone. The stereochemistry at C-6 center is determined by configuration of the aldehyde which is derived from either L-glutamic acid or D-glutamic acid. The (2S, 3S) or (2R, 3R) β-hydroxy DAP isomers were not available by this condensation. Many methods exist for inverting the configuration of a hydroxy group in alcohols.[151,152] They usually involve activation of the hydroxy group followed by an S_N2 substitution by another hydroxy-equivalent group (e.g. Mitsunobu reaction). Since the normal aldol adduct **24** (or **27**) was always contaminated by the rearranged compound **25** (or **28**) in which the hydroxy group was benzoylated, these methods could not be used. Although it was well precedented that some boron-enolates, in contrast to corresponding lithium-enolates, could undergo aldol condensations with *anti* stereoselectivity,[153,154] the boron, magnesium or titanium enolates of **22** (or **23**) did not alter the relative stereoselectivity of the Seebach condensation with aldehydes.[121]

A variety of literature methods are available for dehydroxyfluorination of alcohols to fluorinated compounds.[111,115] Among them, diethylaminosulfur trifluoride (DAST) has been widely utilized. In our research group, it was shown that DAST converts a series of protected β-hydroxy amino acids to corresponding β-fluoro analogs, that upon deprotection give the free β-fluoro amino acids (Figure 17).[155]

Figure 17 Dehydroxyfluorination of β-Hydroxy Amino Acid to β-Fluoro Amino Acid by the Action of DAST.



P, P', P" = protecting groups

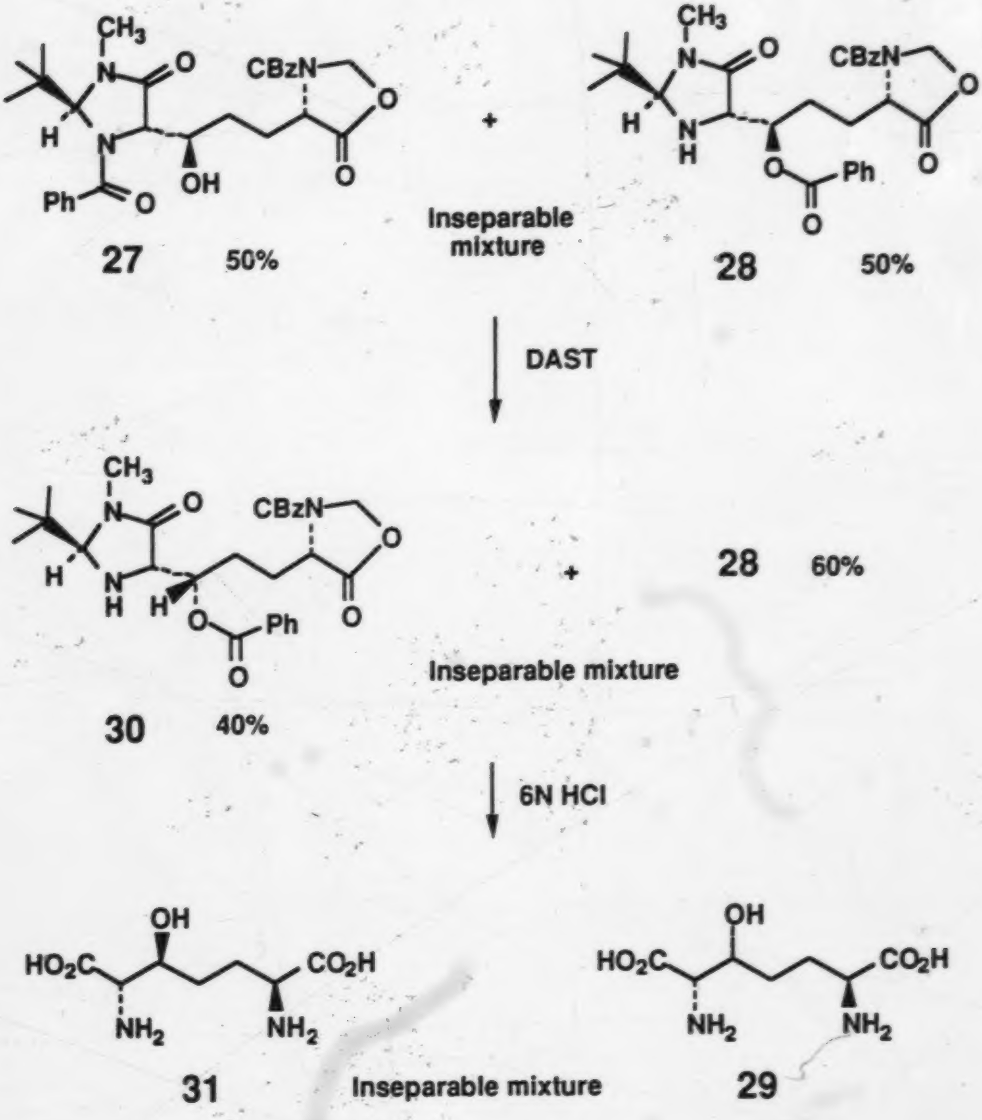
To convert the aldol adduct **27** to the corresponding fluoro compound, the mixture of **27** and **28** (50 : 50 ratio) was treated with DAST in CH_2Cl_2 at -78°C. It was anticipated that the aldol adduct **27** would be dehydroxyfluorinated while the rearranged isomer **28** would remain unreacted; separation of the product from **28** would provide access to the desired compound. However, no trace of the desired fluoro derivative was detected. Analysis of the product by ^{1}H NMR and MS spectrometry demonstrated that the reaction yields the adduct **28** as well as another O-benzoyl isomer **30**, which possesses the opposite configuration at C-3, in a ratio of 40 : 60 (Scheme 9).

The stereochemical assignment for compound **30** was confirmed by subsequent hydrolysis of the mixture of **30** and **28** to the corresponding β-hydroxy DAPs in a ratio of 40 : 60 (Scheme 9). The major isomer was found to be identical by NMR spectrometry to isomer **29** obtained earlier. The minor isomer was subsequently identified as having the structure **31** by an independent route as described later (Scheme 17).

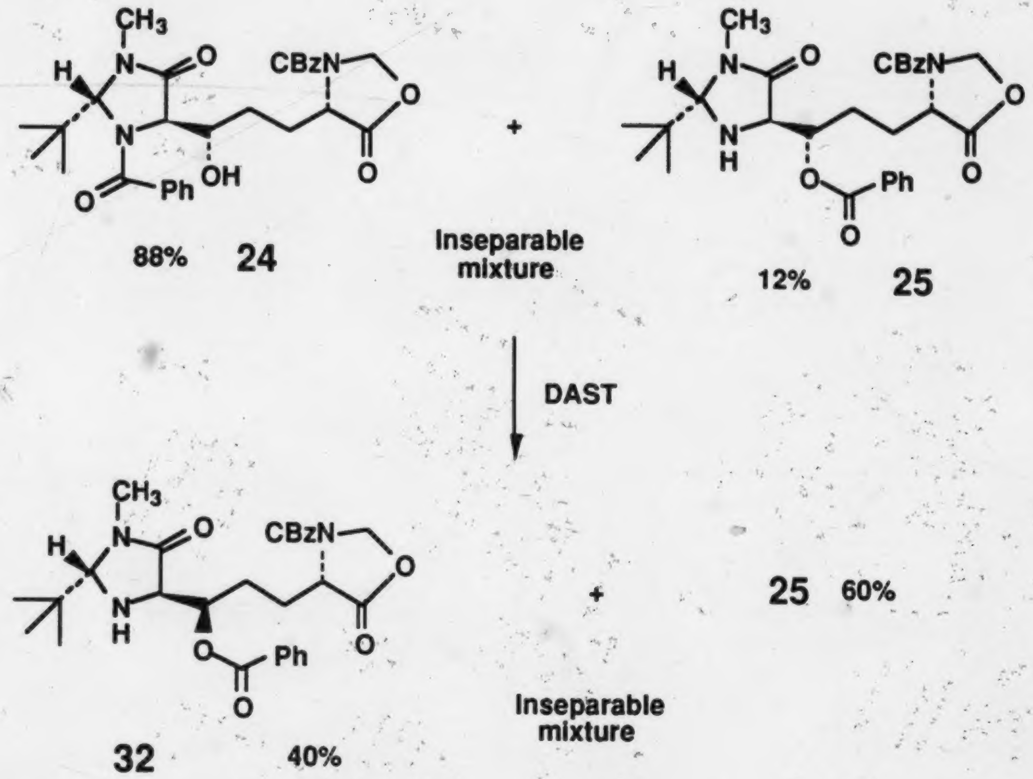
The mixture of **24** and **25** (88 : 12) was treated analogously with DAST. Interestingly, based on ^{1}H NMR analysis, a mixture of compounds **32** and **25** was obtained in the same ratio (40 : 60) (Scheme 10), even though the starting material ratios for the normal aldol adduct and the rearranged derivative were different (88 : 12 *vs* 50 :50).

The probable mechanism for these DAST-mediated transformations involves the formation of a five-membered heterocyclic intermediate **32b** (Scheme 11). This presumably undergoes rapid epimerization at the C-5 center via the carbocation **32c** to reach an equilibrium, where the two epimers of **32b** are in a ratio of 40 : 60. Upon aqueous work-up, **32b** generates the mixture of **32** and **25** (or **30** and **28**) in the same product ratio. Similar amide group involvement during DAST dehydroxyfluorination has also been observed by other investigators.[156-159]

Scheme 9

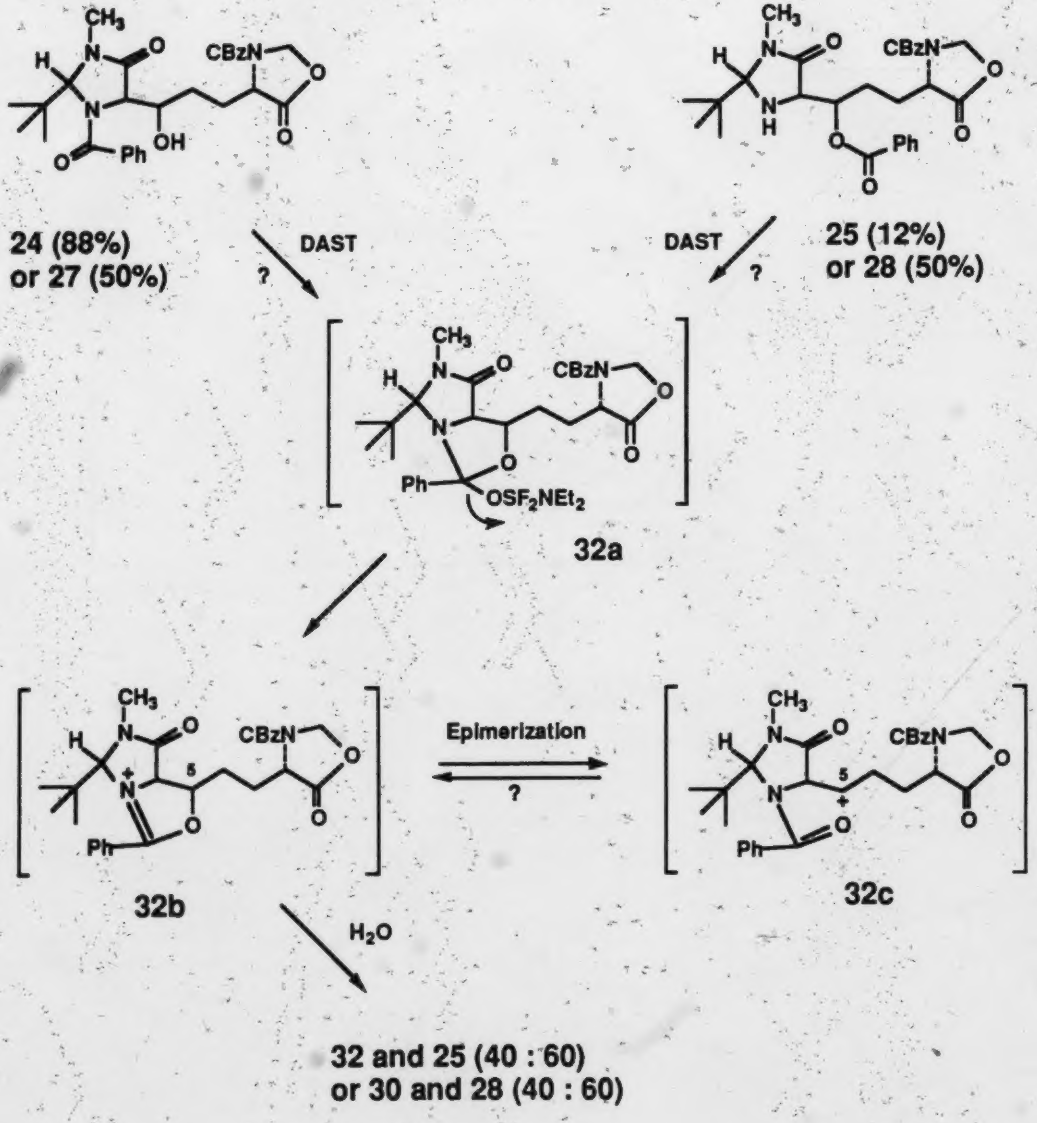

27 50%
Inseparable mixture
28 50%
DAST
30 40%
28 60%
Inseparable mixture
6N HCl
31
Inseparable mixture
29

Scheme 10


88%
24
Inseparable mixture
12%
25
DAST
32
40%
Inseparable mixture
25
60%

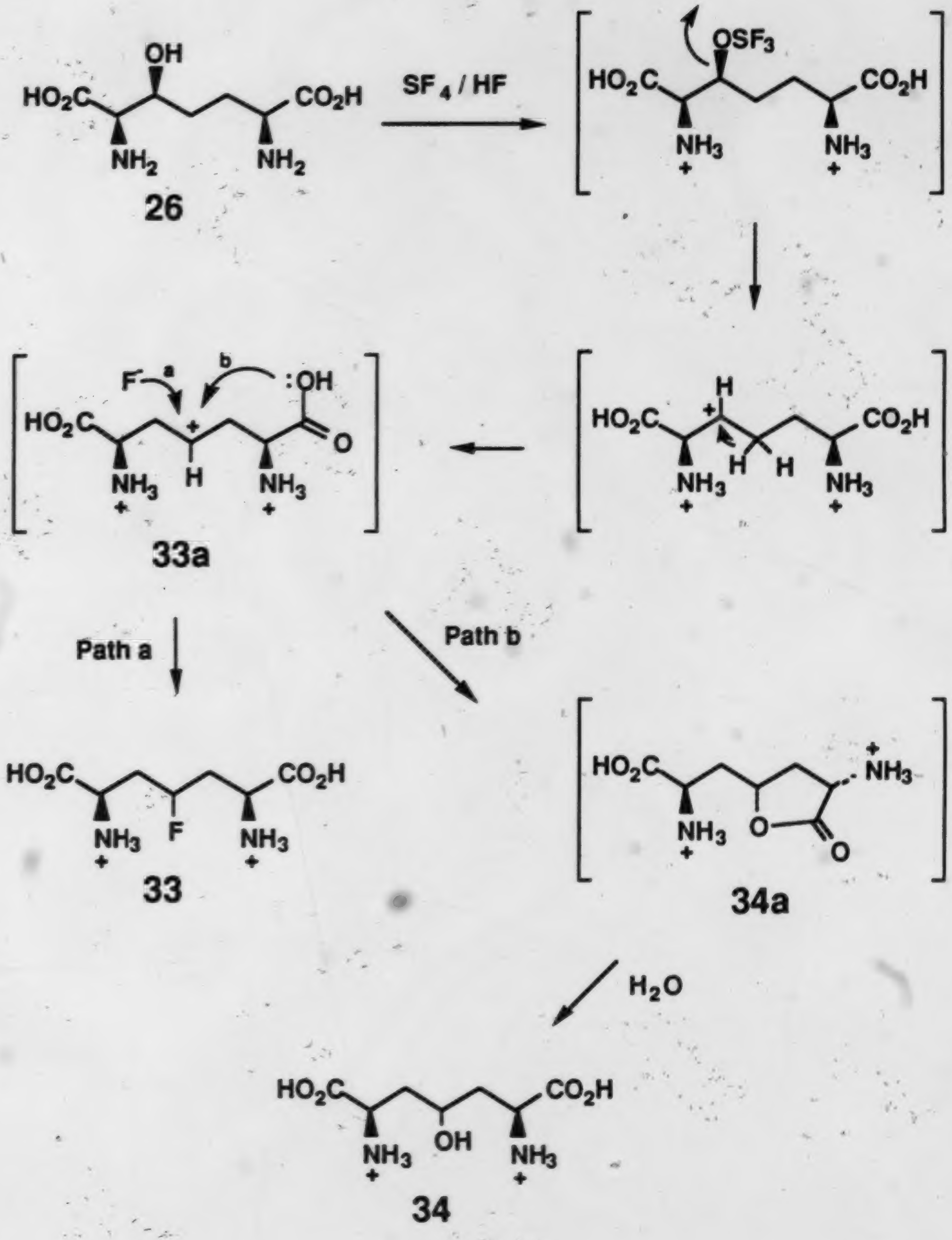
It was apparent that if the isomer **30** (or **32**) could be separated from the isomer **28** (or **25**), hydrolysis of **30** (or **32**) would provide the β-hydroxy DAP isomer that was not available from the Seebach condensation. Unfortunately, the separations by flash chromatography and preparative TLC were not possible and resulted in extensive decomposition of the products.

Scheme 11


24 (88%)
or 27 (50%)
DAST
?
DAST
?
25 (12%)
or 28 (50%)
32a
Epimerization
?
32b
32c
H_2O
32 and 25 (40 : 60)
or 30 and 28 (40 : 60)

Since reaction of the Seebach aldol adducts with DAST did not provide access to the desired β-fluoro DAP, alternative approaches were sought. A variety of free β-hydroxy amino acids are converted to corresponding β-fluoro derivatives by treatment with liquid hydrogen fluoride and sulfur tetrafluoride.[115] It was anticipated that reaction of β-hydroxy DAP **24**, **29** or **5** with liquid HF and SF_4 would afford the desired β-fluoro DAP directly. To test this, the β-hydroxy DAP isomer **26** was dissolved in liquid HF and SF_4 was introduced into the solution at -78°C (Scheme 12). Purification of the product by ion exchange chromatography, reverse phase MPLC, and preparative TLC were unsuccessful, but, two major compounds were separated in almost equal amounts by HPLC.[117] Mass spectrometry indicated that one of the products is a fluoro DAP and the other is a hydroxy DAP. Additional evidence for the existence of the fluoro DAP came from ^{19}F NMR spectroscopy. From a preliminary ^{1}H NMR study of the fluorinated compound, it was clear that it was not the desired β-fluoro DAP because the proton at C-2 should give a doublet of doublets due to coupling with both the fluorine and hydrogen on the adjacent carbon (C-3), but this was absent. Moreover, ^{1}H NMR spectra of the hydroxy DAP were fundamentally different from those of the starting β-hydroxy DAP **26**. The ^{13}C NMR spectrum of the hydroxy DAP displayed only four peaks, indicating that there were three pairs of equivalent carbons in the molecule. Based on these observations, the compound is a symmetric γ-hydroxy DAP **34** instead of a β-hydroxy DAP. The ^{13}C NMR spectrum of the fluoro DAP also shows four different carbons rather than seven once carbon-fluorine couplings are considered. This confirms the fluorinated product is the symmetric γ-fluoro DAP **33** (Scheme 12). ^{1}H NMR analysis confirms these structural assignments of **33** and **34**.

Scheme 12



SF4 / HF
HO2C
OH
CO2H
NH2
OSF3
NH3
F
:OH
a
b
H
O
Path a
Path b
H2O

26

33a

33

34a

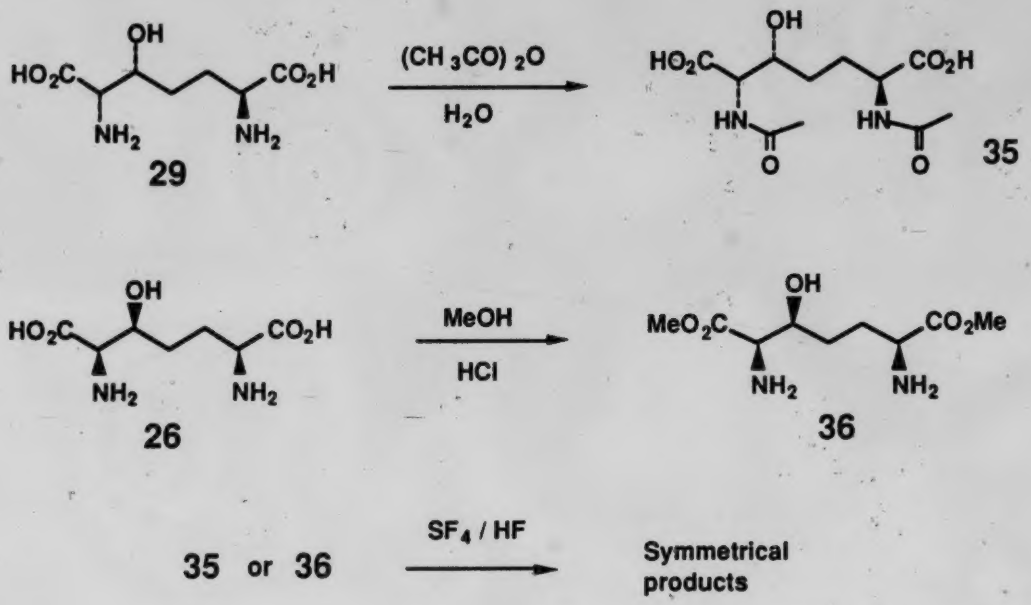
34

The mechanism for formation of **33** and **34** from **26** with HF /SF_4 probably involves a hydride migration (Scheme 12). Activation of the hydroxy group by SF_4 followed by elimination forms a carbocation in which the positive charge resides at the β-position. Nucleophilic capture of the carbocation by fluoride would lead to formation of the desired β-fluoro DAP. However, the carbocation is apparently very unstable under the acidic conditions (HF/SF_4) wherein all the amino groups and carboxylic groups are presumably protonated. Repulsion between the positive charge at the β-position and the nearby protonated groups may force the former charge to relocate to the γ-position, by hydride migration (Scheme 12). Capture of a fluoride from the reaction medium by the γ-carbocation **33a** affords the γ-fluoro DAP **33**.

Under the anhydrous conditions employed during the fluorination of **26**, it is unlikely that the γ-hydroxy DAP **34** could arise by reaction of carbocation **33a** with water. Intramolecular participation of the carboxylic acid group at the positively charged central carbon could generate γ-lactone **34a**, which would give γ-hydroxy DAP **34** upon work-up with aqueous acid. Use of DAST, a milder fluorinating reagent than SF_4, gave only recovered starting material.

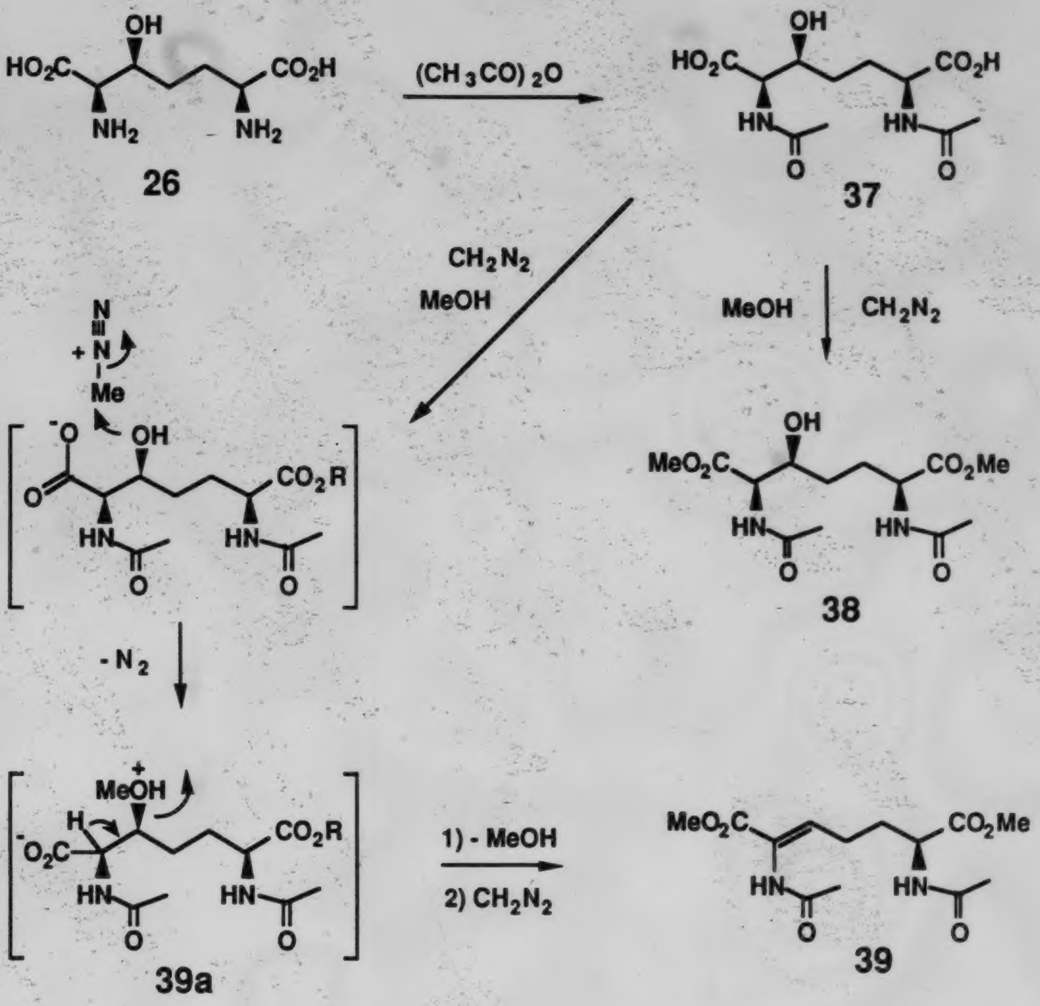
It seemed that if the nitrogen atoms in **26** were less prone to protonation, the tendency of the β-carbocation to rearrange to the γ-carbocation would be reduced and formation of β-fluoro compound should be more favorable. To achieve this, β-hydroxy DAP **29** was treated with acetic anhydride to generate the N, N'-diacetyl-β-hydroxy DAP **35** in 97% yield (Scheme 13). However, reaction of **35** with HF/SF_4 fails to give the desired β-fluoro derivative. The ^1H NMR spectrum of the crude product displays a signal pattern similar to that of **34** which is indicative of a symmetric structure. The β-hydroxy DAP dimethyl ester **36**, which was easily made by the action of anhydrous methanol saturated with HCl gas in quantitative yield, was also subjected to reaction with HF/SF_4. Again, no desired β-fluoro compound was produced and the unwanted rearrangement still predominated.

Scheme 13



The fully protected β-hydroxy DAP **38** was prepared by esterification of N, N'-diacetyl β-hydroxy DAP (**37**) with diazomethane in 71% yield (Scheme 14). A side-product **39** was also isolated in 7% yield. The dehydration may be rationalized as protonation of diazomethane by the carboxylic acid, which was followed by methylation of hydroxy group rather than the carboxylate to generate intermediate **39a** which undergoes loss of methanol to afford **39** (analogous hydroxyl methylations by diazomethane were also observed by other investigators).[60,61] Fully protected derivative **38** also fails to circumvent the rearrangement problem with SF_4/HF.

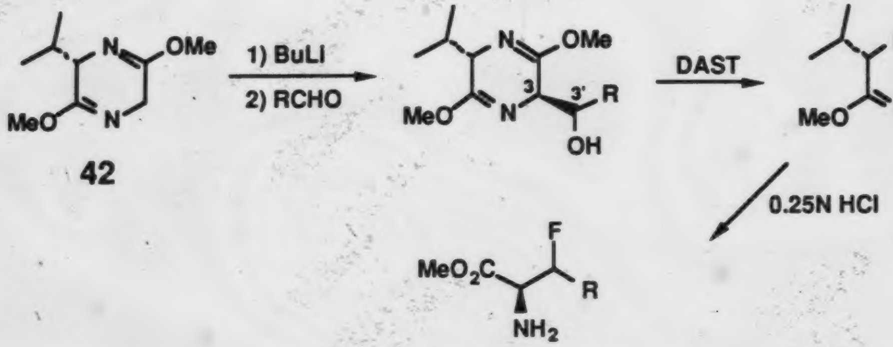
Scheme 14



OH
HO2C
CO2H
NH2
NH2
26
(CH3CO)2O
37
HN
O
CH2N2
MeOH
MeOH
CH2N2
N
+ N
Me
-O
O
CO2R
MeO2C
CO2Me
38
- N2
MeOH
H
-O2C
39a
1) - MeOH
2) CH2N2
39

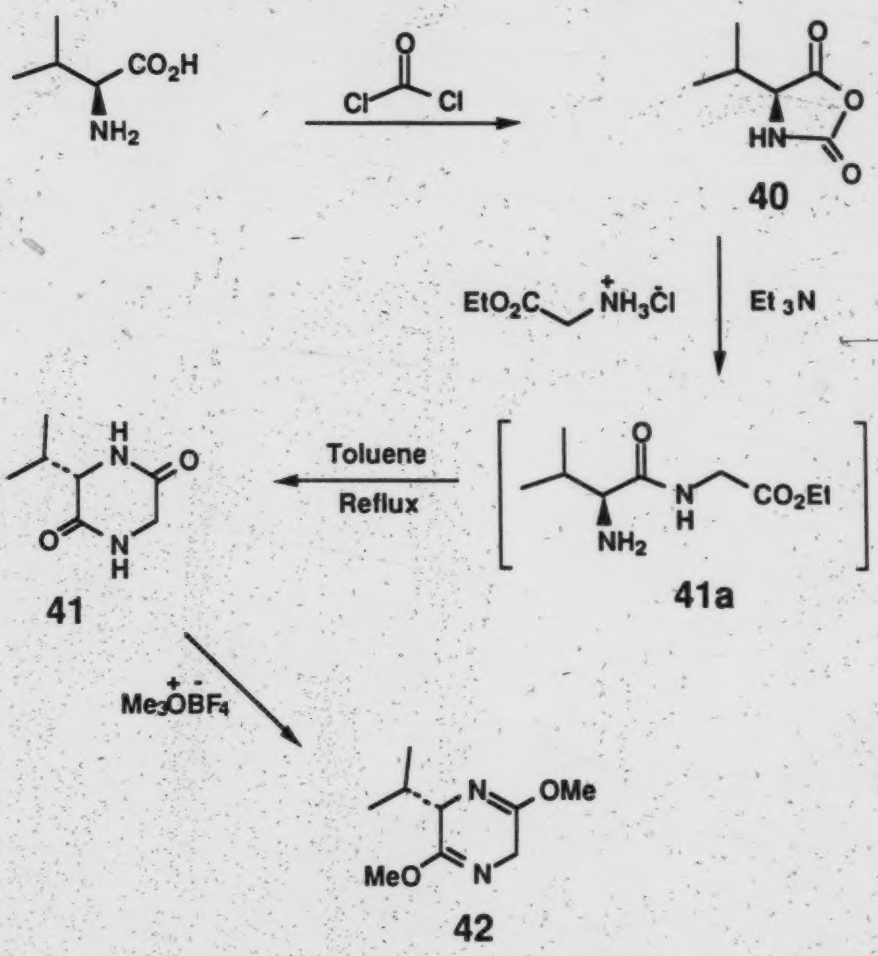
The synthetic studies described above, demonstrated that a new approach to β-fluoro DAP was necessary. According to Schoellkopf and co-workers, aldol adducts derived from condensation between a chiral bislactim ether (e.g. **42**) and aldehydes or ketones could be transformed to the corresponding fluoro derivatives by the action of DAST (Figure 18).[162] Since aldehyde **15** was available, aldol reaction with the bislactim ether **42** followed by the DAST fluorination and removal of protecting groups should provide access to the desired β-fluoro DAP isomers.

Figure 18 Synthesis of β-Fluoro Amino Acid by Schoellkopf's Condensation Followed by DAST Treatment.



To obtain the bislactim ether **42**, L-valine N-carboxy anhydride **40** (available from the treatment of L-valine with phosgene in quantitative yield) was condensed with ethyl glycinate to give the ethyl L-valine-glycinate dipeptide **41a** (Scheme 15).[163,164] Without purification, the ring closure of the dipeptide to form **41** was effected (in 29% yield) by heating to reflux in dry toluene. This diketopiperazine was alkylated with trimethyloxonium tetrafluoroborate to afford **42** (95% yield) with an optical purity close to the literature value.[164]

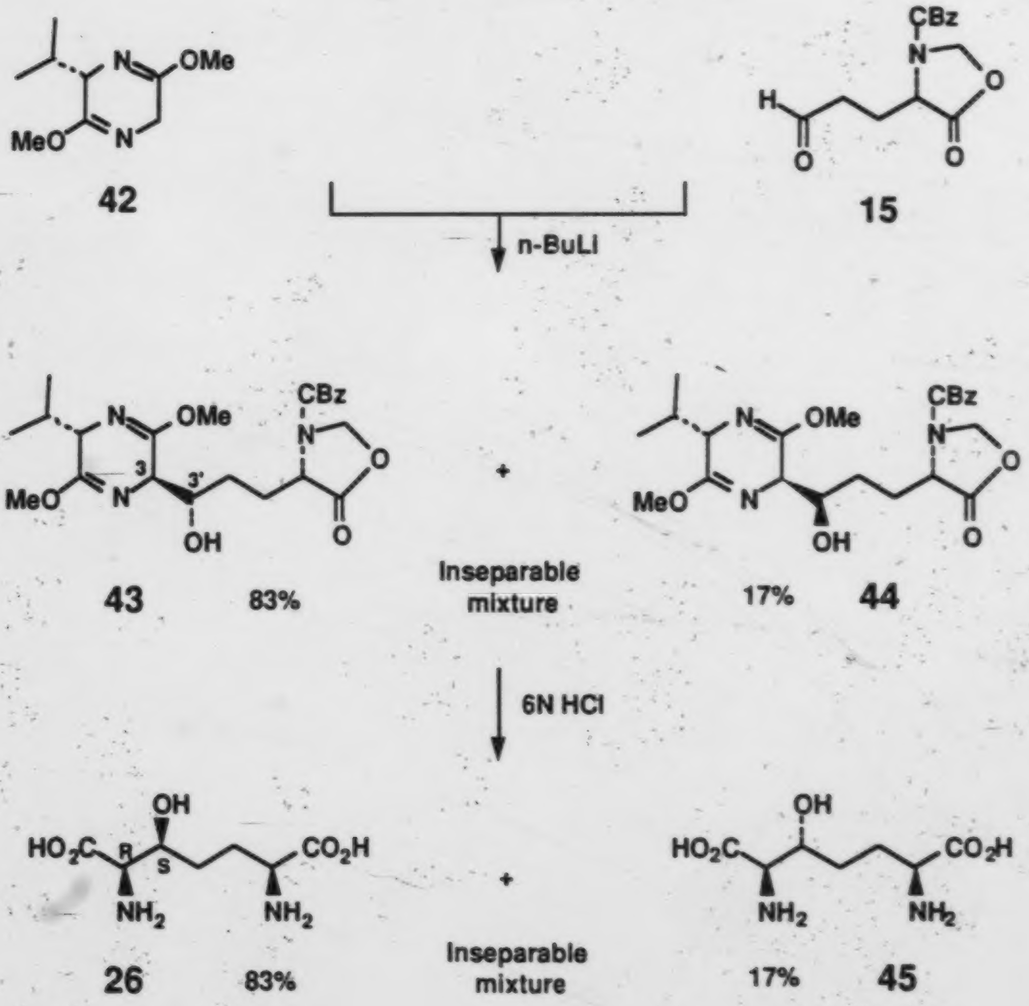
Scheme 15


CO$_2$H
NH$_2$
Cl
Cl
O
O
HN
O
O
40
EtO$_2$C
NH$_3$Cl
Et$_3$N
Toluene
Reflux
H
N
O
O
N
H
41
O
N
H
CO$_2$Et
NH$_2$
41a
Me$_3$OBF$_4$
N
OMe
MeO
N
42

As expected, aldol condensation between the bislactim ether **42** and aldehyde **15** offered the desired adducts in 52% yield (Scheme 16). Analysis of the products by ^{1}H NMR indicated that two diastereomers **43** and **44** in a ratio of 83 : 17 were produced, which were epimers at the hydroxy center (C-3'). Because of the steric hindrance imposed by the isopropyl group, the incoming aldehyde preferentially adds to the enolate *anti* to it. In agreement with literature reports,[118] the stereoselectivity at C-3 is very high (d.e. >95%)

and the two diastereomers **43** and **44** possess the expected stereochemistry at C-3, but differ at the C-3' center. Unlike the Seebach condensation discussed before, the Schoellkopf condensation does not provide high stereoselectivity at C-3' center (d.e. 10-85%).[118] In this case, the diastereomeric excess (d.e.) was 66%. An effort to separate the two diastereomers **43** and **44** by means of silica gel MPLC chromatography failed, as

Scheme 16



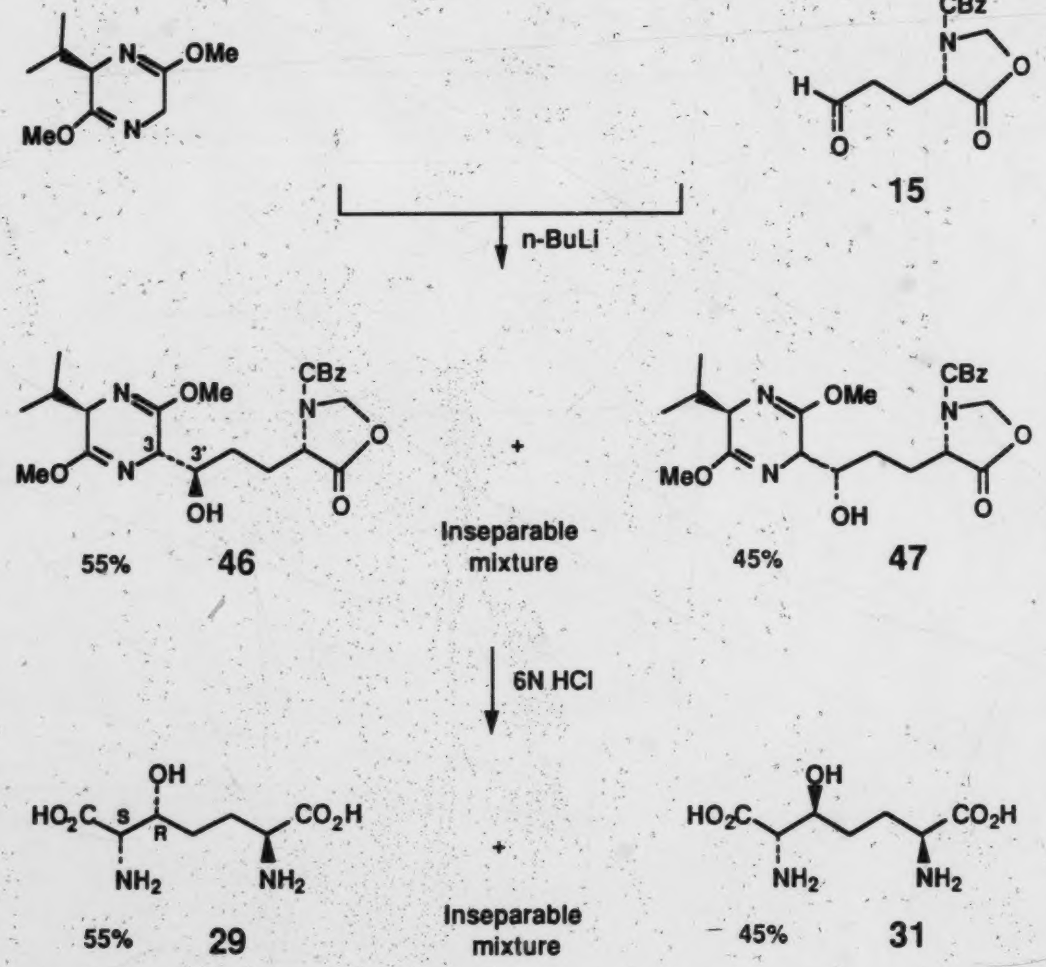
42
15
n-BuLi
43
83%
Inseparable mixture
17%
44
6N HCl
26
83%
Inseparable mixture
17%
45

did attempted derivatization of the alcohols to corresponding O-silyl ethers with t-butyldimethylsilyl chloride.

In order to determine the configurations of the two epimers **43** and **44** at C-3', the mixture of the aldol adducts was hydrolyzed in 6N HCl, and a mixture of the two corresponding β-hydroxy DAP isomers **26** and **45** (99% overall yield) thus formed were separated from the by-product, L-valine, by cellulose chromatography. As expected, ^{1}H NMR and ^{13}C NMR spectra of the mixture did show distinctive peaks for the two isomers. To determine their absolute stereochemistry, pure β-hydroxy DAP isomer **26**, synthesized earlier by the Seebach condensation, was added to the mixture. The intensity of the peaks belonging to the major isomer (83%) were shown to be increased upon the addition. Accordingly, the minor isomer **45** (17%) was assigned as the hydroxy epimer of **26**. Like the Seebach aldol condensation, the Schoellkopf aldol reaction performed here also favored the 2R, 3S isomer, although to less extent, in agreement with observations by Schoellkopf and co-workers.[118]

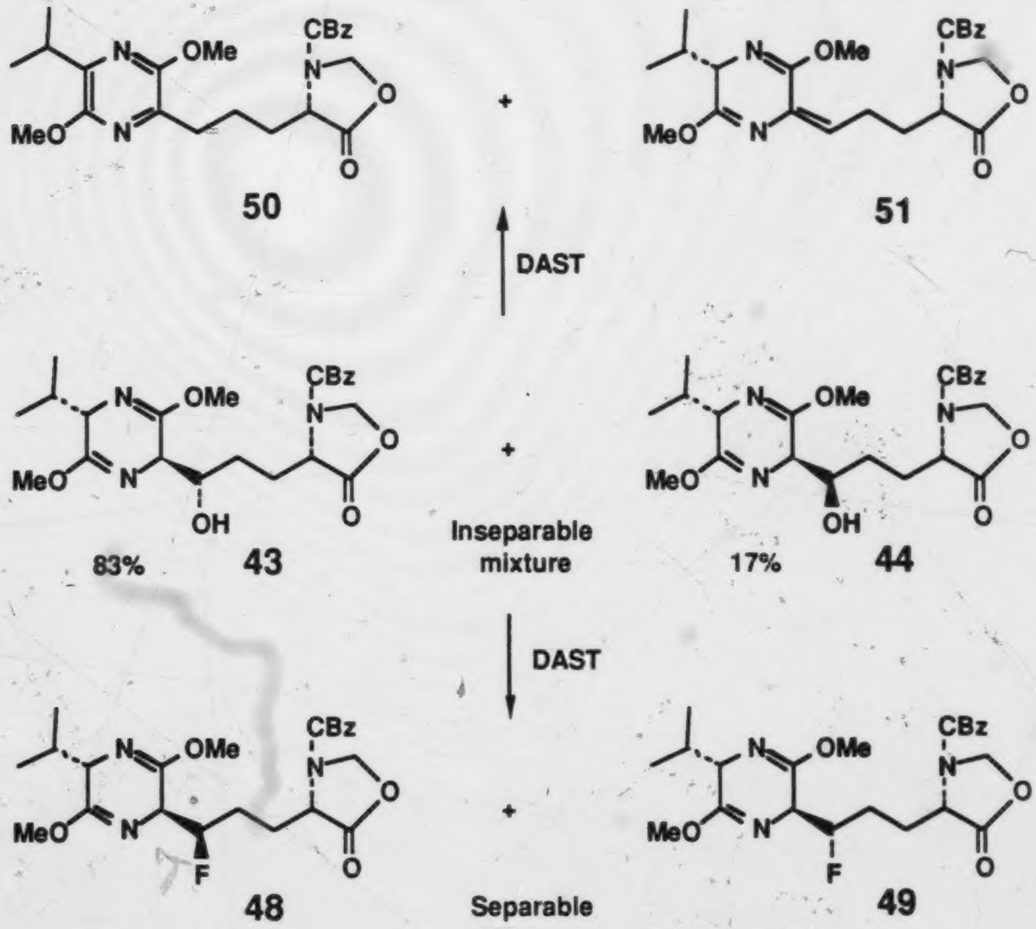
Similarly, the bislactim ether derived from D-valine, upon enolization with n-BuLi, reacts with aldehyde **15** to produce aldol adducts **46** and **47** in a ratio of 55 : 45 in 35% yield (Scheme 17). In this case, the stereoselectivity at C-3' was even poorer (d.e. 10%). Since the transition states of the two aldol condensations are diastereomeric, it is not surprising that the stereoselectivities at C-3' are different (83 :17 *vs* 55 : 45). Subsequent hydrolysis of the adducts **46** and **47** as a mixture in 6N HCl again yielded two β-hydroxy DAP epimers (57% overall yield) whose stereochemistries were determined by the NMR technique described above. Addition of isomerically pure **29**, synthesized previously by the Seebach condensation, to the NMR sample tube of the mixture shows that the major isomer (55%) is identical to it.

Scheme 17

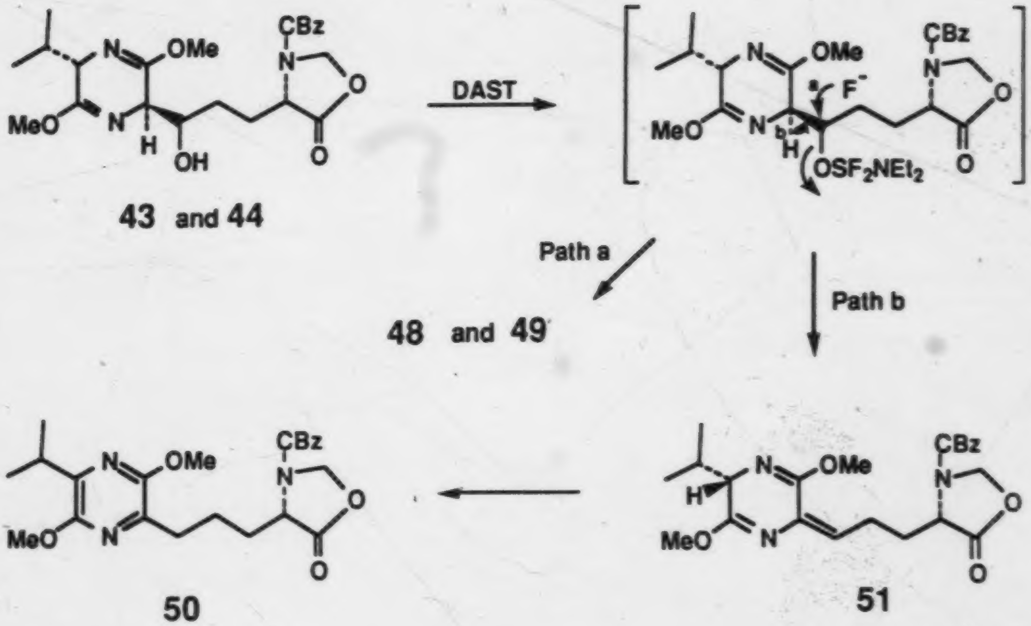

OMe
MeO
CBz
H
O
15
n-BuLi
3
3'
OH
55%
46
+
Inseparable mixture
45%
47
6N HCl
HO_2C
S
R
CO_2H
NH_2
55%
29
45%
31

Dehydroxyfluorination of a mixture of aldol adducts **43** and **44** with DAST provides the desired fluoro compounds **48** and **49**, but in a very low yield (15%) (Scheme 18). A dehydrated compound **51**, as well as an aromatized compound **50**, were also isolated in 30% and 10% yields, respectively. Similar dehydration phenomena associated with DAST fluorination were also observed by other investigators.[148,162] Although the two fluoro isomers **48** and **49** were not separable by flash chromatography, they could be resolved on a silica gel MPLC column. A possible mechanism for the DAST fluorination is illustrated in Scheme 19. The competition between fluorination (path a) and dehydration (path b) in favor of the latter suggests that the elimination process is kinetically faster than the substitution because of the acidity of the C-3 proton. Morpholinosulfur trifluoride

Scheme 18


OMe
CBz
MeO
50
+
51
DAST
43
83%
Inseparable mixture
17%
44
OH
DAST
48
F
Separable
49

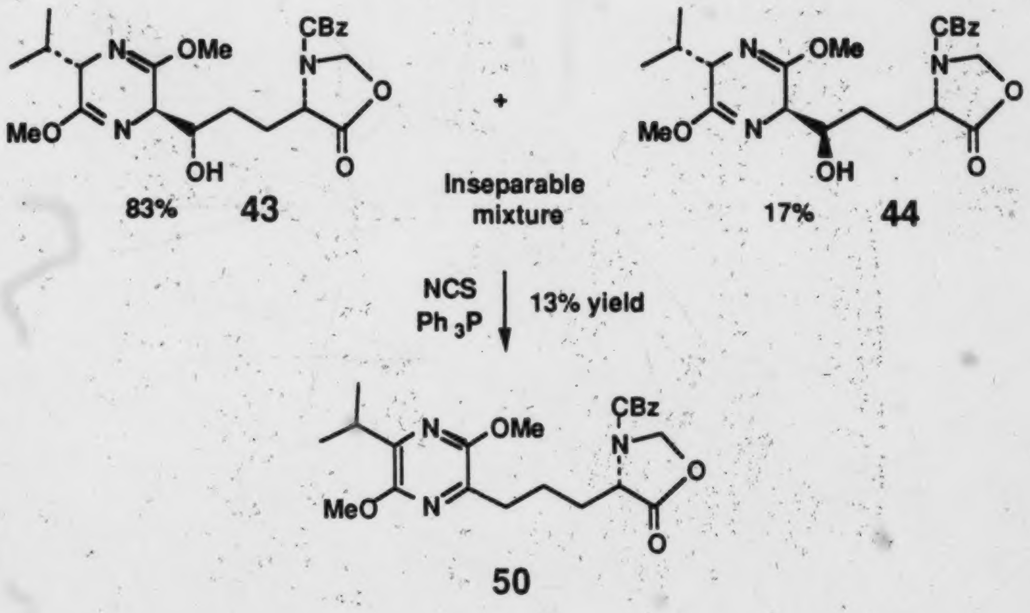
Scheme 19


CBz
N
O
OMe
MeO
H
OH
43 and 44
DAST
F⁻
a
b
OSF2NEt2
Path a
Path b
48 and 49
50
51

(Morpho-DAST) is claimed to be an ideal fluorinating reagent that produced less dehydration side reactions than normal DAST.[150,165] However, treatment of the mixture of aldol adducts **43** and **44** with morpho-DAST gave the same result as DAST.

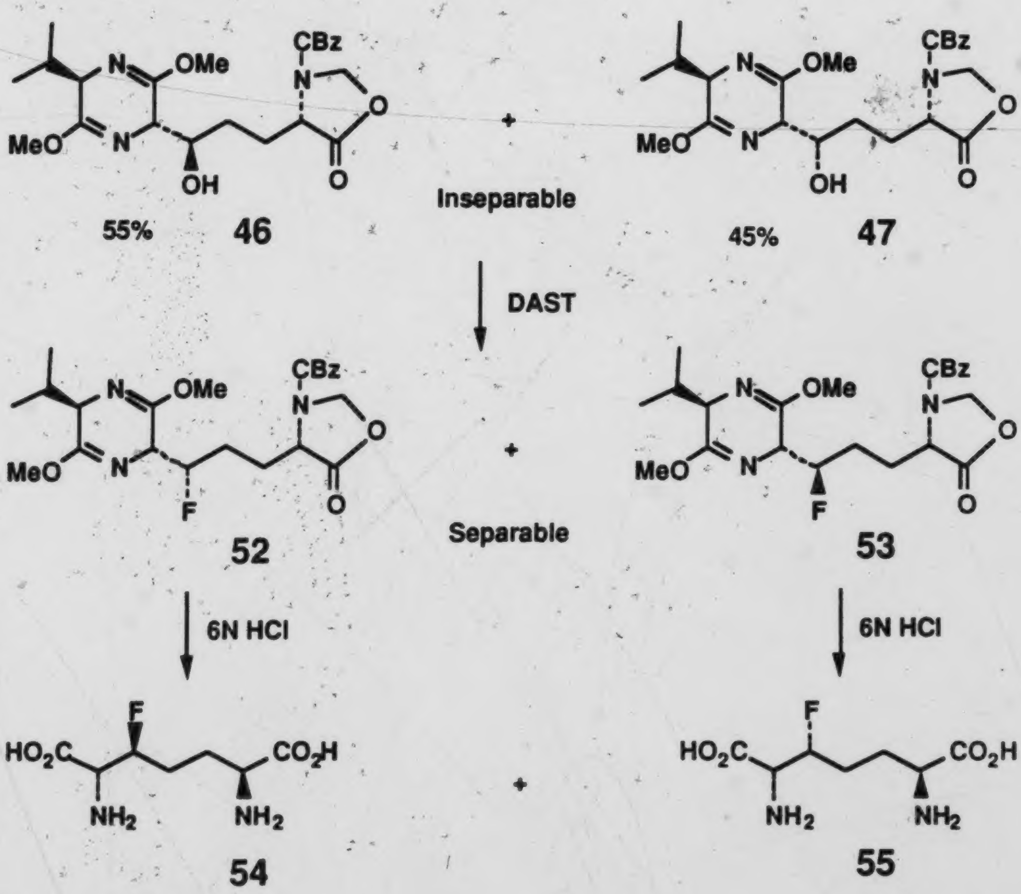
Treatment of mixture of **43** and **44** with N-chlorosuccinimide and triphenyl phosphine produces (Scheme 20) a complex mixture, from which the aromatized compound **50** was isolated in 13% yield. No desired β-chloro compounds could be detected.

Scheme 20



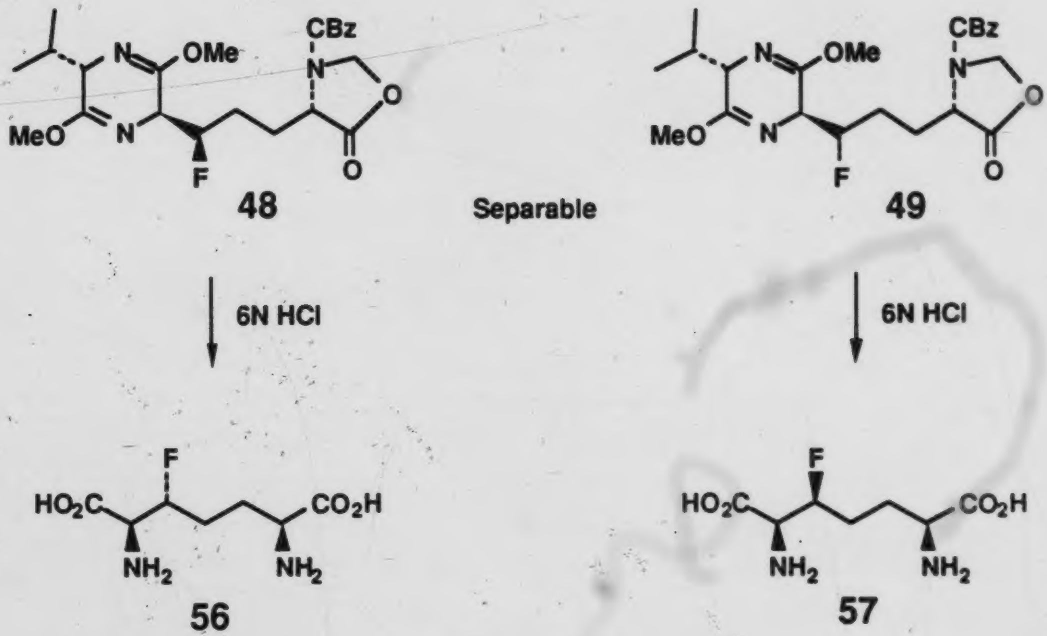
The mixture of aldol adducts **46** and **47** derived from (2R)-bislactim ether were analogously fluorinated with DAST (Scheme 21). Two fluoride isomers **52** and **53** were separated from each other by silica gel MPLC chromatography, although the purification of isomer **53** from other impurities was very difficult. Despite the low yield (8.3%) and dehydration problems experienced with this DAST reaction, subsequent hydrolyses of isomer **52** and isomer **53** (impure) in 6N HCl, followed by extensive purification with the use of cellulose chromatography were able to provide the intended β-fluoro DAP isomers **54** (47% yield) and **55** (2.3% overall yield from **46** and **47**) in stereochemically pure form.

Scheme 21


55%
46
Inseparable
45%
47
DAST
52
Separable
53
6N HCl
6N HCl
54
55

Isomers **48** and **49** (impure) were also separately hydrolyzed in 6N HCl (Scheme 22). Although attempts to purify the resulting crude products by means of ion exchange chromatography, reverse phase chromatography, and HPLC failed, extensive purification with cellulose chromatography afforded the two pure β-fluoro DAP isomers **56** (51% yield) and **57** (0.8% overall yield from **43** and **44**).

Scheme 22



As discussed previously, the stereochemistry of aldol adducts **43**, **44**, **46** and **47** is known; but the configurations of the fluoro compounds **48**, **49**, **52** and **53** had to be determined by an independent method. Beguin and co-workers[166] introduced a method for configurational determination of β-fluoro-α-amino acids. In their paper, *erythro* and *threo* configurational assignments had been made for eleven β-fluoro-α-amino acids or esters by employing the effect of complexation of the protonated amino group by 18-crown-6 ether on ^{19}F NMR and ^{1}H NMR parameters. For the *erythro* configurations, coupling constants between α-proton and β-fluorine $^3J_{HF}$ increase and coupling constants between α- and β-protons $^3J_{HH}$ decrease upon addition of the crown ether. The opposite effects occur for the *threo* configurations. Application of this method to the β-fluoro DAP isomers gives only a small $^3J_{HF}$ increase (0.37 Hz) for isomer **56** (*erythro*) and $^3J_{HH}$ remains unchanged. For isomer **54**, no measurable $^3J_{HF}$ increase or $^3J_{HH}$ decrease occurs. This may be due to the presence of two amino acid functionalities which could both complex with the crown ether, whereas the β-fluoro-α-amino acids previously examined contain only one amino acid group. Transformation of β-fluoro glutamic acids to rigid five membered lactams allows determination of the relative configurations by measurement of $^3J_{HH}$ or $^3J_{HF}$,[167] but similar approach with β-fluoro DAPs was not practical.

Examination of the literature on ^{1}H NMR data of β-fluoro-α-amino acids,[115,167-169] shows that coupling constants between α- and β-protons $^3J_{HH}$ for *erythro* β-fluoro-α-amino acids are smaller than those for the corresponding *threo* isomers (Table 1). The structures were determined by X ray crystallography or by other independent methods. ^{1}H NMR coupling constant measurements on the β-fluoro DAP isomers indicated that $^3J_{HH}$ for the major isomers **54** and **56** were 2.4 Hz in each case whereas those for the minor isomers **55** and **57** were 4.8 and 4.4 Hz, respectively. Therefore the two major isomers are assigned *erythro* configuration and the two minor isomers are assigned *threo* configuration. The stereochemical assignment of the β-fluoro DAPs together with product ratios for the DAST reactions (Scheme 18 and 21) shows that the DAST fluorination proceeds via an inversion of configuration.

Further comparison of literature data[153] demonstrates that this coupling constant trend is also true for β-hydroxy-α-amino acids. The $^3J_{HH}$ data are listed in Table 2. As expected, the spectra and stereochemical assignments of the β-hydroxy DAP isomers **26**, **29**, **31** and **45** synthesized earlier are in agreement with these observations.

Table 1 Comparison of Coupling Constants $^3J_{HH}$ of *Erythro* and *Threo* β-Fluoro α-Amino Acids.

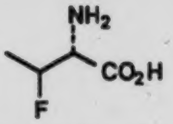
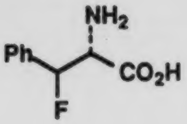
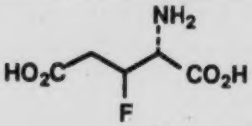
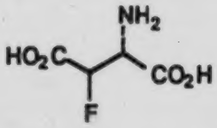
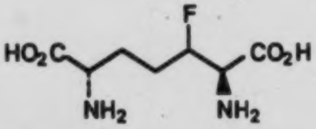
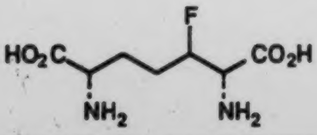
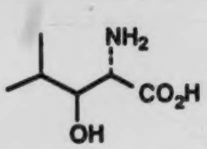
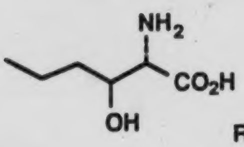
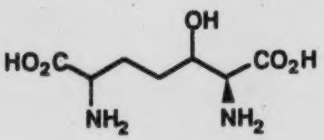
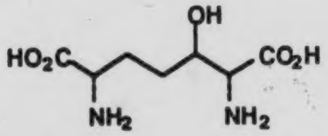
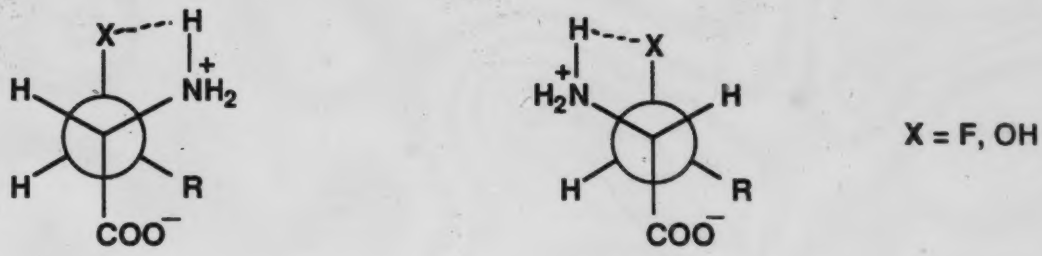
β-Fluoro amino acid	$^3J_{HH}$ (Hz)	
	Erythro	Threo
 Ref. 115	2.0	3.0
 Ref. 168	3.4	4.3
 Ref. 167	2.6	3.9
 Ref. 115 Ref. 169	2.0	2.0
	2.4	4.8
	2.4	4.4

Table 2 Comparison of Coupling Constants $^3J_{HH}$ of *Erythro* and *Threo* β-Hydroxy α-Amino Acids.

β-Hydroxy amino acid	$^3J_{HH}$ (Hz)	
	Erythro	**Threo**
 Ref. 153	2.7	3.6
 Ref. 153	3.6	4.5
	3.5	5.2
	4.8	5.2

Since the ^1H NMR coupling constant between the key hydrogens is governed by their dihedral angle, the net coupling constant is a weighted average contributed by all possible conformers. Hydrogen bonding of the fluorine with adjacent amino group may cause *erythro* and *threo* β-fluoro-α-amino acids to prefer one conformation over another (Figure 19). Similarly, hydrogen bonding between the hydroxy group and adjacent amino group of β-hydroxy-α-amino acids could favor the conformations in which the *erythro* isomer shows a smaller coupling constant than the corresponding *threo* isomer.

Figure 19 Possible Hydrogen Bonding in *Erythro* and *Threo* Isomers of β-Fluoro Amino Acids or β-Hydroxy Amino Acids.

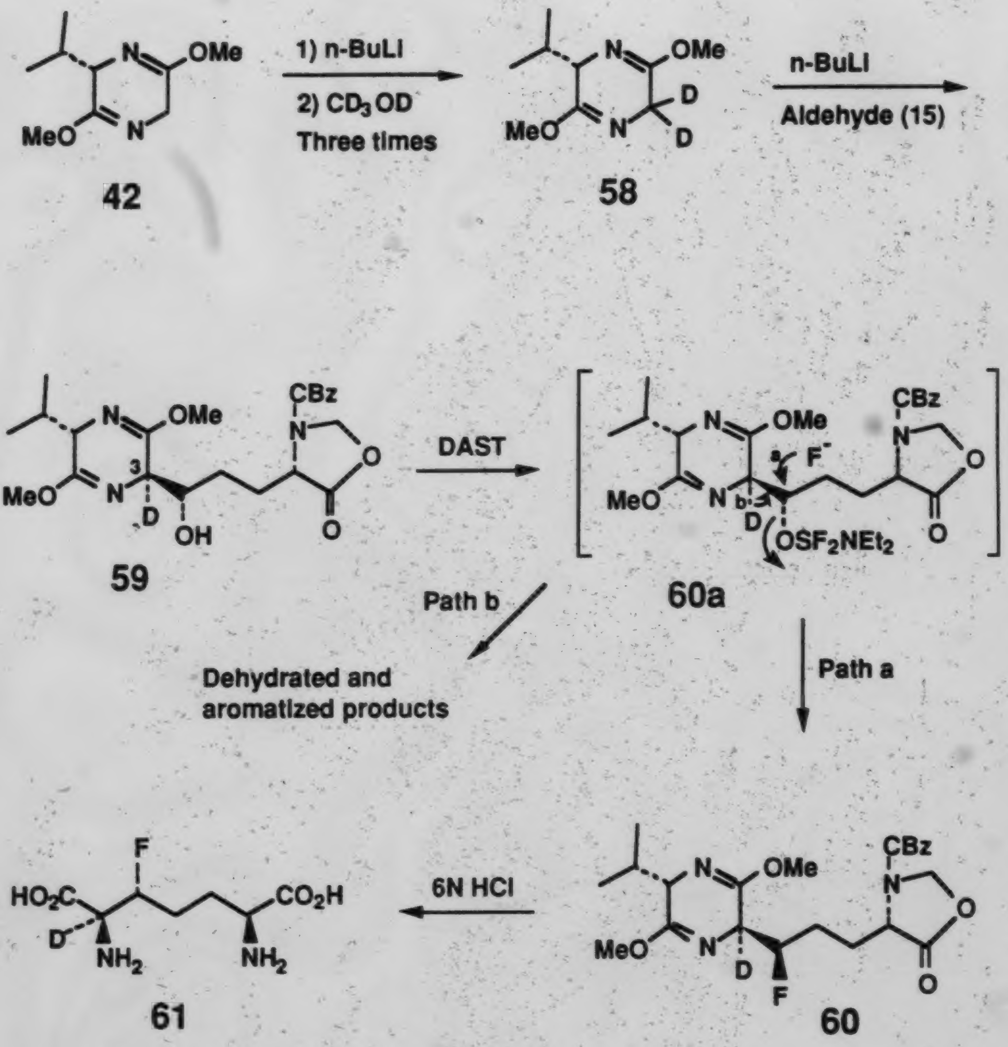


erythro

threo

Enzyme kinetic isotope studies required the α-deutero-β-fluoro DAP isomer **61**, which was synthesized as shown in Scheme 23. To prepare the doubly deuterated (2S) bislactim ether **58**, the bislactim ether **42** was enolized with n-BuLi, quenched with CD_3OD, and then this process was repeated twice. The ^1H NMR and MS spectra of **58** show that more than 98% bisdeuteration was achieved, and that there was no non-deuterated species remaining. Due to an isotope effect, the preference for proton abstraction to deuterium loss during the enolization process was larger than 4 to 1. Condensation of the deuterated bislactim ether **58** with aldehyde **15** gave **59** (35% yield), which was dehydroxyfluorinated by DAST. Interestingly, the presence of deuterium in **59** helps suppress the dehydration side reaction during the fluorination, as shown by ^1H NMR and

Scheme 23


1) n-BuLi
2) CD₃OD
Three times
42
58
n-BuLi
Aldehyde (15)
59
DAST
60a
Path b
Path a
Dehydrated and aromatized products
6N HCl
60
61

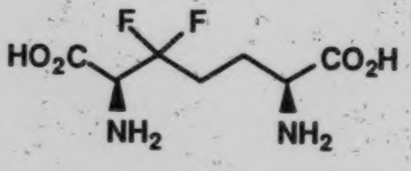
TLC. This may be due to an isotope effect that hinders deprotonation of **59** relative to **43** (Scheme 19). After separation of the two fluoro compounds as before, the major isomer **60** was hydrolyzed in 6N HCl and separated by cellulose chromatography to afford pure α-deutero-β-fluoro DAP (**61**) (4.5% overall yield from **59**).

In summary, two β-hydroxy DAP isomers **26** and **29** were synthesized in stereochemically pure form utilizing the Seebach aldol condensation. More importantly, the Schoellkopf aldol condensation followed by DAST fluorination allows the synthesis of stereochemically pure isomers **54**, **55**, **56**, and **57** of β-fluoro DAP. Enzyme inhibition studies with DAP epimerase will be discussed later.

Attempted Synthesis of β, β-Difluoro Diaminopimelic Acid Isomers

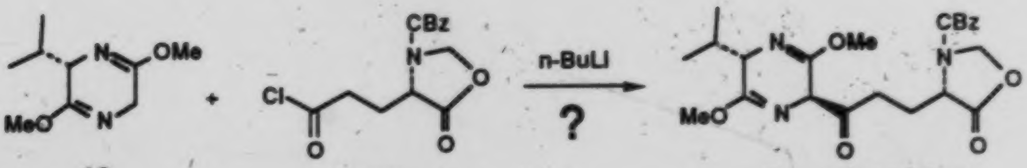
With completion of the synthesis of four β-fluoro DAP isomers, the next target was β,β-difluoro DAP (Figure 20). This compound appeared to possess interesting potential for inhibition of DAP enzymes, if control of the stereochemistry at the two amino centers could be achieved.

Figure 20 (2S, 6S) β,β-Difluoro Diaminopimelate.



One possible synthetic route to the desired compound involves an acylation of the bislactim ether **42** with acyl chloride **14** (Scheme 24). The resulting ketone on treatment with DAST could afford the corresponding difluorinated substance because such transformations of ketones are well documented.[111] Although successful acylations of the bislactim ether with some acyl chlorides had been reported by Schoellkopf and co-workers,[170] the acylation of the lithiated bislactim ether with acyl chloride **14** yields a complex mixture. Although the mass spectrum suggests that the desired ketone may be present in small quantities, attempted purification failed to provide any of the target compound.

Scheme 24



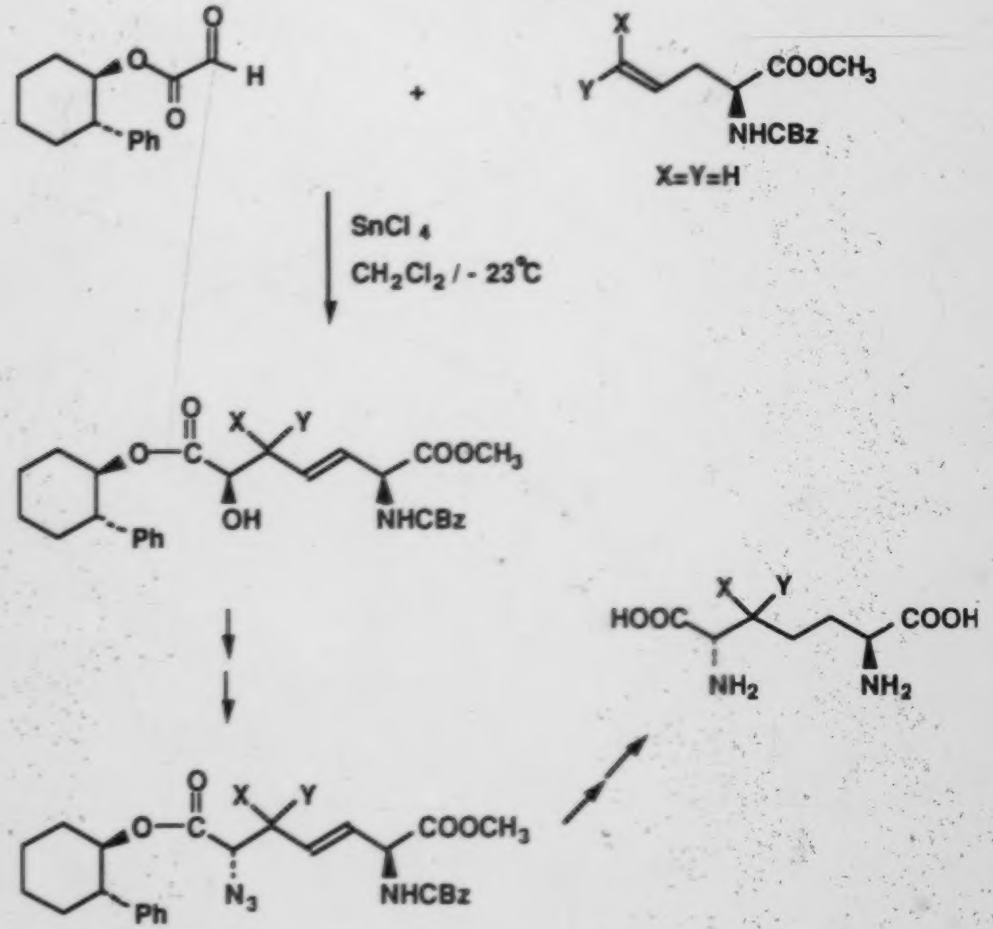
42 14

A complex mixture

An entirely different approach arose through a new synthetic method for making diaminopimelic acid isomers that was developed in our group.[171,172] This approach employs an ene reaction between a chiral glyoxylate ester and methyl allylglycinate (Figure 21, X=Y=H) to generate an olefinic alcohol as shown in Figure 21. This is then converted to its trifluoromethanesulfonate and substituted with NaN_3 to give the corresponding azide. Hydrogenation simultaneously reduces the azide group and the olefinic double to produce a pure DAP isomer. The stereochemical purity of the products is good because both the ene reaction and the following substitution reaction are highly stereoselective.

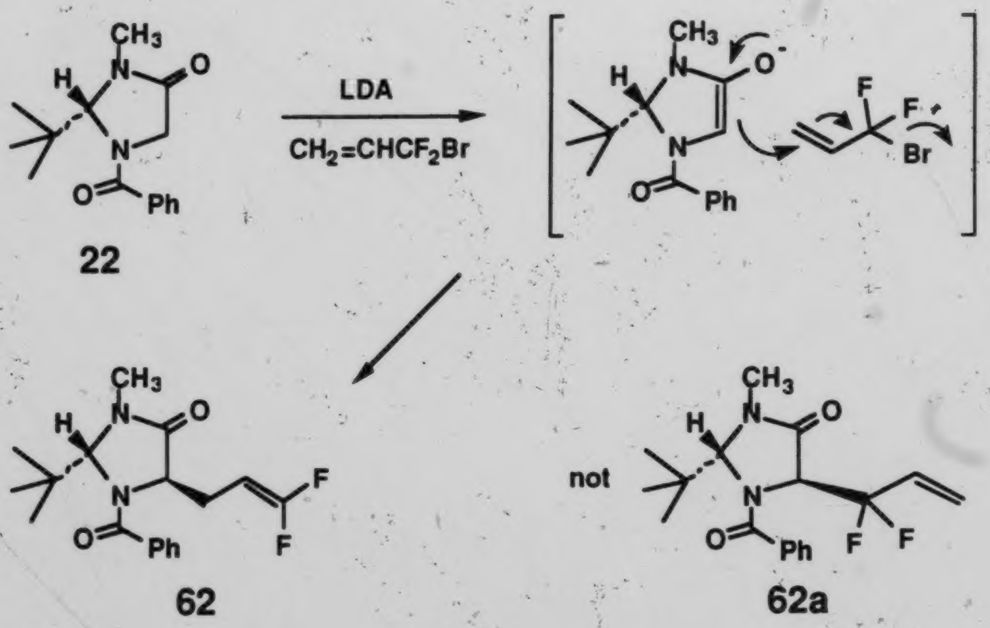
It appeared that if this ene reaction between the chiral glyoxylate ester and methyl difluoroallylglycinate (Figure 21, X=Y=F) were successful, then we should be able to synthesize the desired difluoro DAP isomers. The required difluoroallylglycinate could be made by alkylation of a chiral glycine-equivalent (e.g. Seebach's or Schoellkopf's auxiliary compounds) with 1, 1-difluoro-3-bromopropene. In addition, trifluoroallylglycine derivatives should be accessible by ring opening of protected serine β-lactone[173] by

Figure 21 Synthetic Route to DAP via Ene Reaction.


O
H
O
Ph
+
X
Y
COOCH$_3$
NHCBz
X=Y=H
SnCl$_4$
CH$_2$Cl$_2$ / - 23°C
O
X
Y
O—C
COOCH$_3$
OH
NHCBz
Ph
HOOC
X
Y
COOH
NH$_2$
NH$_2$
O
X
Y
O—C
COOCH$_3$
N$_3$
NHCBz
Ph

trifluorovinyl lithium or magnesium[174,175] compounds. While the synthesis of 1,1-difluoro-3-bromo propene[176] and the β-lactone ring opening reaction were being investigated, 3, 3-difluoro-3-bromopropene was found to be commercially available. It seemed that this bromide might be able to alkylate the Seebach imidazolidinone enolate via an S_N2' rather than S_N2 mechanism. Hence, the enolate of **22** and 3,3-difluoro-3-bromopropene were condensed (Scheme 25).[178-180] As expected, a S_N2' reaction occurred to generate **62** in 51% yield. The diastereoselectivity of the alkylation at the new chiral center was very high and no trace of the other possible isomer was detected by ^{1}H NMR and ^{13}C NMR spectroscopy. It is interesting to note that the chemical shifts for the vinyl proton and the vinyl carbon bearing it are at very high field (δ_H=3.75 and δ_C=71.22) relative to normal vinyl protons and carbons.[181] In contrast, the vinyl carbon bearing the two fluorines gives a doublet of doublets at δ_C =156.9. The evidence for the production of product **62** rather than product **62a** is that only one vinyl proton is observed in the ^{1}H NMR spectrum of **62** rather than three and that it is a doublet of triplets of doublets due to coupling with the *trans* and *cis* fluorines (J_{HF}(trans) = 24.8 Hz, J_{HF}(cis) = 2.0 Hz) as well as with the two adjacent methylene protons.

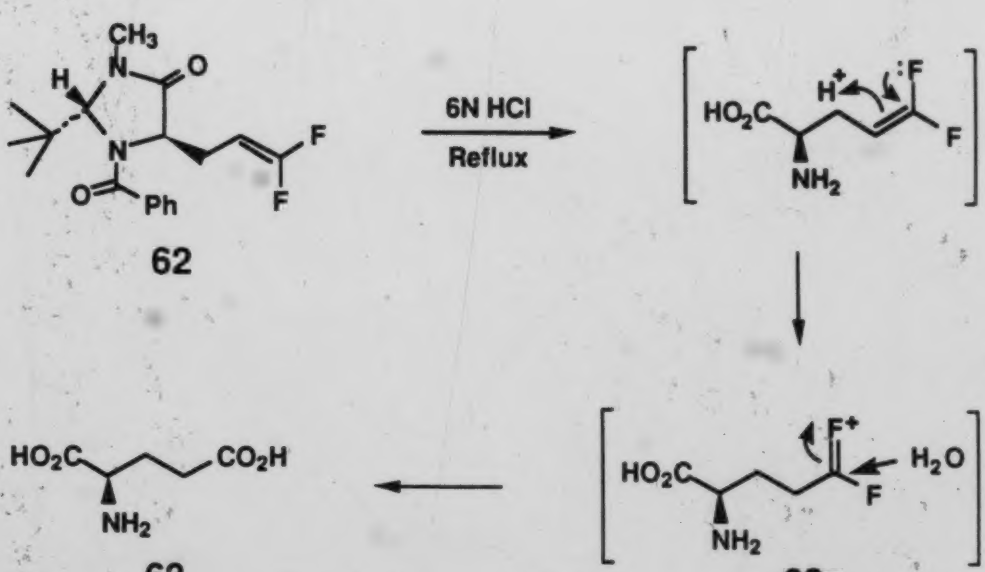
Scheme 25



Compound **62** was hydrolyzed in an attempt to obtain difluoroallylglycine. However, reaction of **62** with 6N HCl at reflux unexpectedly gave D-glutamic acid (**63**) in

48% yield (Scheme 26). A possible mechanism for the formation of **63** may involve protonation of the double bond, followed by addition of water and fluoride loss.

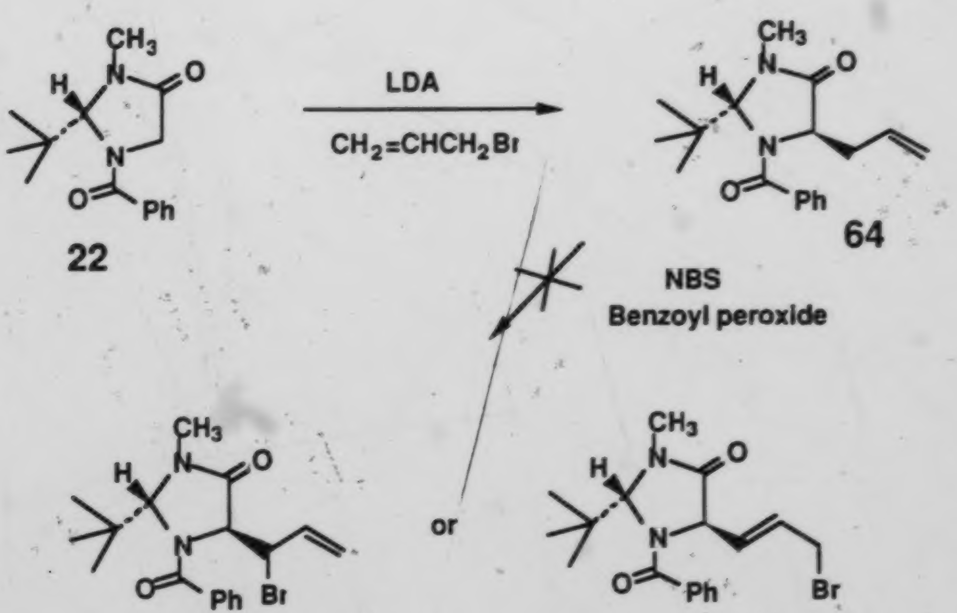
Scheme 26



Attempts were made to epoxidize the double bond in compound **62** in the hope that the resulting epoxide would undergo ring opening by another glycine-equivalent enolate to produce a β-difluoro γ-hydroxy DAP derivative. Unfortunately, all attempts at epoxidation of **62** by *m*-chloroperbenzoic acid or by sodium hypobromite[182,183] failed. It also appeared that if an allylic bromide could be made from **62** by a bromination reaction, subsequent attack with another glycine-equivalent enolate might produce a precursor for the desired β, β-difluoro DAP via either S_N2 or S_N2' substitution. A model bromination reaction with N-bromosuccinimide in the presence of benzoyl peroxide as a radical initiator was attempted[184] on the allylic compound **64**, which was prepared by the alkylation of

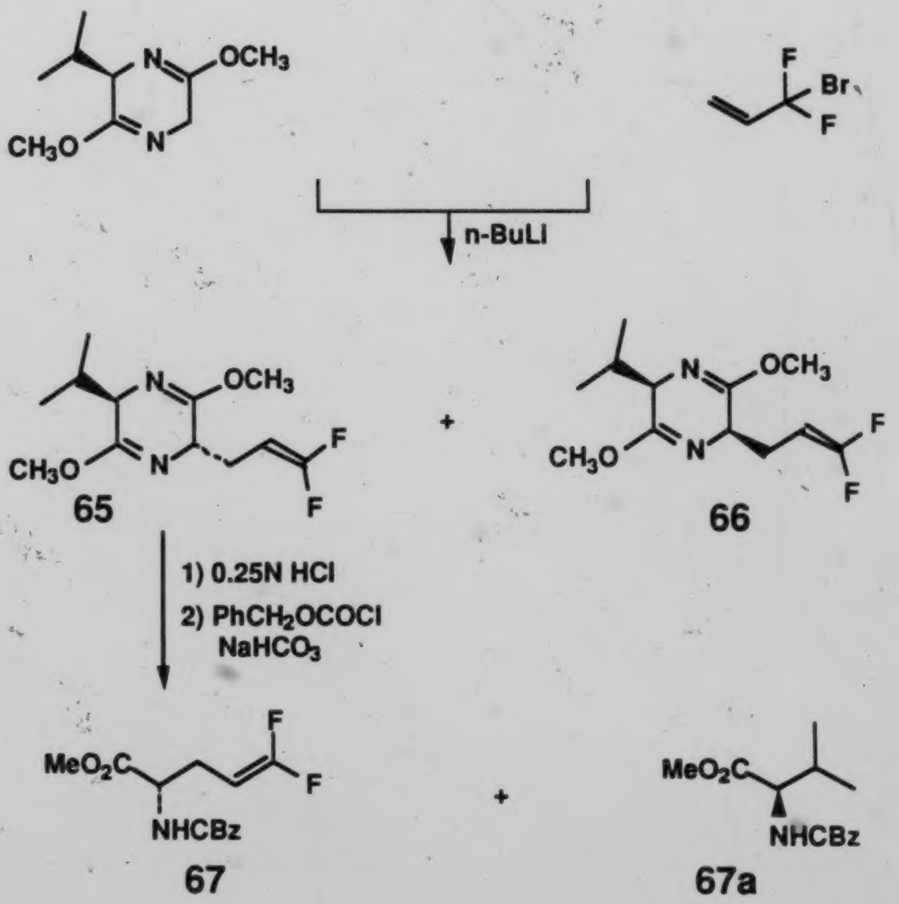
Seebach's imidazolidinone **22** with allyl bromide in 76% yield (Scheme 27). However, only starting material was isolated from all bromination attempts.

Scheme 27



Since the difluorovinyl moiety can not endure strong hydrolysis conditions, as seen in the hydrolysis of **62**, the alkylation of the Schoellkopf bislactim ether with 3, 3-difluoro-3-bromopropene was attempted (Scheme 28). Bislactim ether derivatives undergo hydrolysis under much milder conditions (0.25N HCl at 20°C). The alkylation proceeds through S_N2' mechanism, but unlike the corresponding reaction with Seebach auxiliary which gave very high diastereoselectivity, the process with the bislactim ether[164] yields two separable diastereomers **65** (60% yield) and **66** (20% yield) in a *anti : syn* ratio of 4 : 1 (ratio detected by ^{1}H NMR). Hydrolysis of the major isomer **65** in 0.25N HCl at room temperature produces the methyl difluoroallylglycinate without affecting the difluorovinyl functionality. Although the free amino acid was also generated if the hydrolysis was prolonged (24 h), the reaction could be controlled to give only the methyl ester by shortening the reaction time to 4 h. Condensation with carbobenzoxy chloride of the crude hydrolysis mixture after basification of the medium with $NaHCO_3$ afforded methyl N-CBz difluoroallylglycinate **67** in 26% yield.

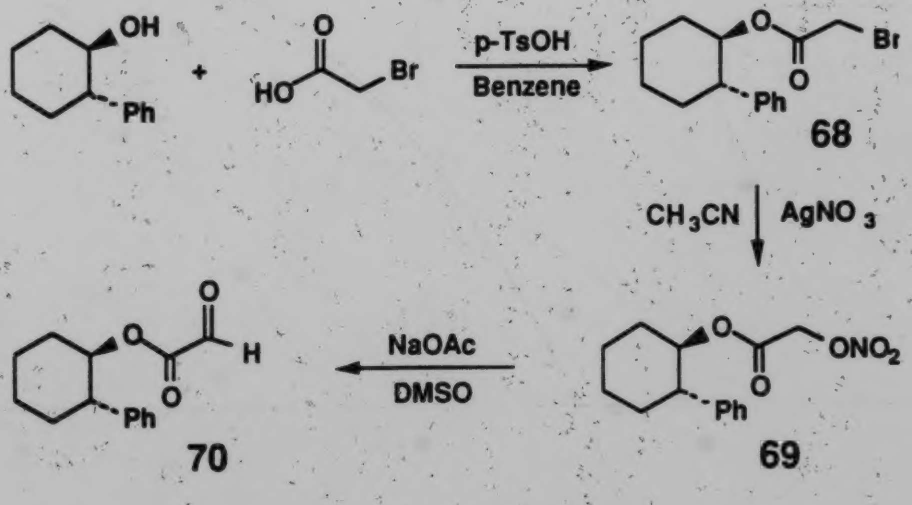
Scheme 28



OCH3
CH3O
N
F
Br
F
n-BuLi
65
66
+
1) 0.25N HCl
2) PhCH2OCOCl
NaHCO3
MeO2C
NHCBz
67
67a

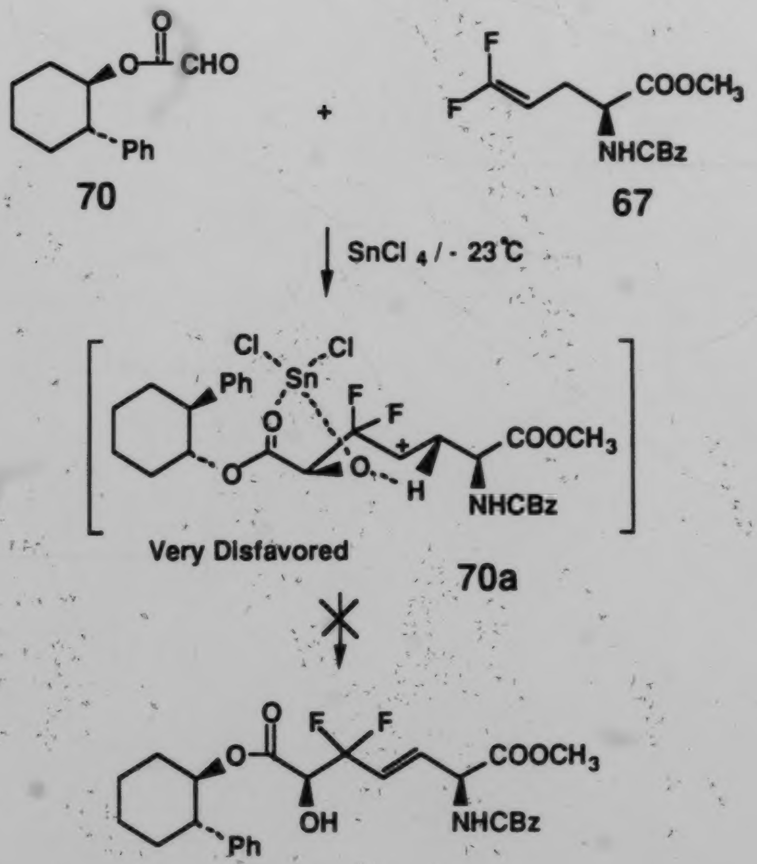
With the difluoro olefin **67** available, the phenylcyclohexyl glyoxylate **70** was prepared by a modified literature procedure (Scheme 29).[185] Optically pure trans-2-phenylcyclohexanol[160,161] condenses with bromoacetic acid in the presence of *p*-toluenesulfonic acid in boiling benzene to afford the bromoacetic ester **68** in quantitative yield. Treatment of **68** with excess silver nitrate gave the nitrate ester **69** in 98% yield, presumably through a silver-assisted substitution of the bromide by nitrate group. The desired glyoxylate **70** could be obtained in 76% yield by the reaction of **69** with dimethyl sulfoxide (DMSO) in the presence of sodium acetate as a weak base. The mechanism for the formation of the aldehyde functionality in the glyoxylate **70** is analogous to Swern oxidation.[230] Since the aldehyde carbonyl group of **70** is highly active, the product was isolated mainly in the hydrated form, as could be seen from the very low peak intensity of the aldehyde proton in the ^{1}H NMR spectrum. Distillation of **70** under vacuum (100-110 °C / 0.025mmHg) not only purified the product but also dehydrated most of it to the free aldehyde. It was also known that free aldehyde could be generated in *situ* from the hydrate form by the action of one equivalent Lewis acid (e.g. $SnCl_4$).[185]

Scheme 29



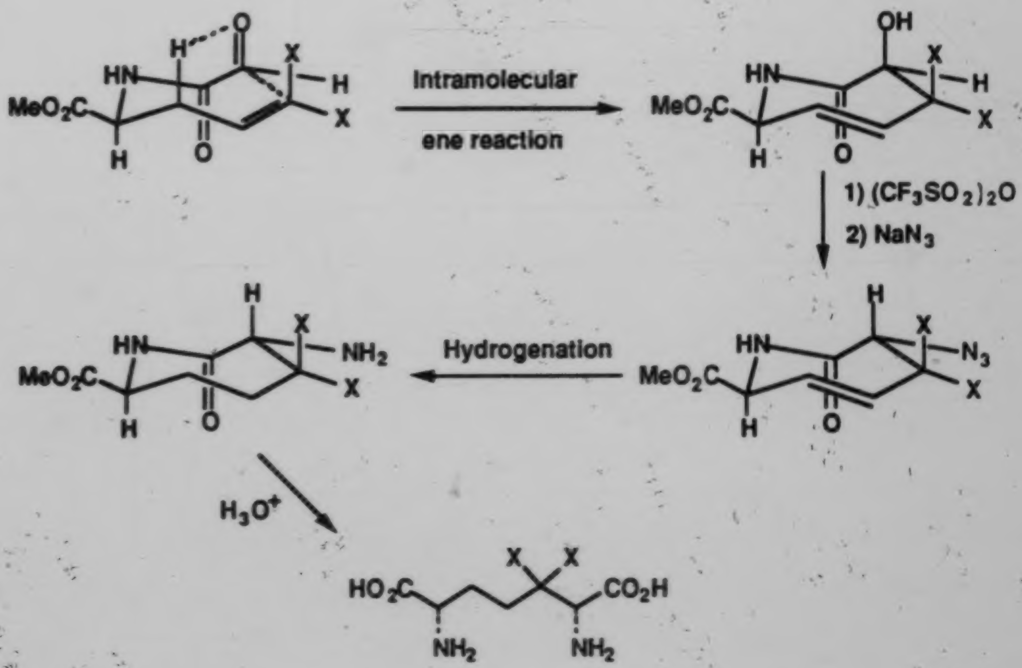
With completion of the synthesis of **67** and **70**, the ene reaction was attempted at –23 °C in CH_2Cl_2 using tin (IV) tetrachloride as a Lewis acid catalyst.[185] Unfortunately, the major compounds isolated from the reaction were the starting materials, glyoxylate **70** and the difluoroallylglycinate **67**. Elevation of the temperature to reflux still gave no reaction. Attempted ene reactions of **70** with compounds **62** and **65** also led to recovery of starting materials under conditions where **70** condenses successfully with a variety of other olefins.

Scheme 30


CHO
Ph
70
+
F
F
COOCH3
NHCBz
67
SnCl4 / - 23 °C
Cl
Cl
Sn
Ph
F
F
COOCH3
H
NHCBz
Very Disfavored
70a
F
F
COOCH3
OH
NHCBz
Ph

Initially, it seemed that the ene reaction would proceed via a concerted mechanism rather than by a stepwise one, and that significant charge separation was unlikely to develop in the transition state. However, failure of the ene reaction with the difluoroalkenes suggests a stepwise process in which a positive charge may develop at the center adjacent to the difluoromethylene carbon (Scheme 30). Since fluorine is highly electron-withdrawing, such a positive charge is likely to be highly disfavored. However, it appeared that if the ene process could occur in an intramolecular[186-192] rather than intermolecular fashion, the reaction might be more favorable. From analysis of molecular models (Figure 22), the desired intramolecular ene reaction was quite promising because the participating atoms and bonds in the molecule were able to form a favorable six-member ring as the transition state. If successful, a seven member lactam would form with high stereospecificity.

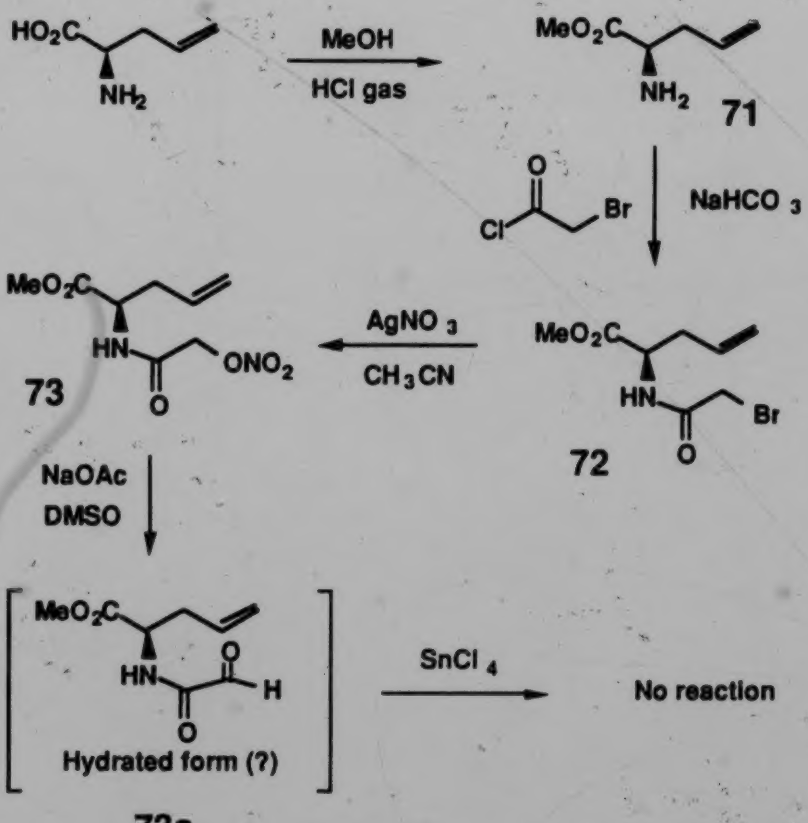
Figure 22 Possible Route to β,β-Difluoro DAP via Intramolecular Ene Reaction.



Subsequent transformation of the hydroxyl to a triflate, substitution with NaN_3, and hydrogenation followed by hydrolysis would provide the desired β, β-difluoro DAP.

Synthesis of the key ene precursor (Figure 22, X=H) and its difluoro analogue (X=F) were attempted simultaneously. To make the compound **73a** (Scheme 31), D-allylglycine was converted quantitatively to its methyl ester **71** with anhydrous methanol saturated with hydrogen chloride gas. N-Bromoacetylation of the ester **71** proceeds with bromoacetyl chloride in aqueous $NaHCO_3$ solution in 90% yield. Treatment of N-bromoacetyl ester **72** with silver nitrate gave the corresponding nitrate **73** in 53% yield.

Scheme 31



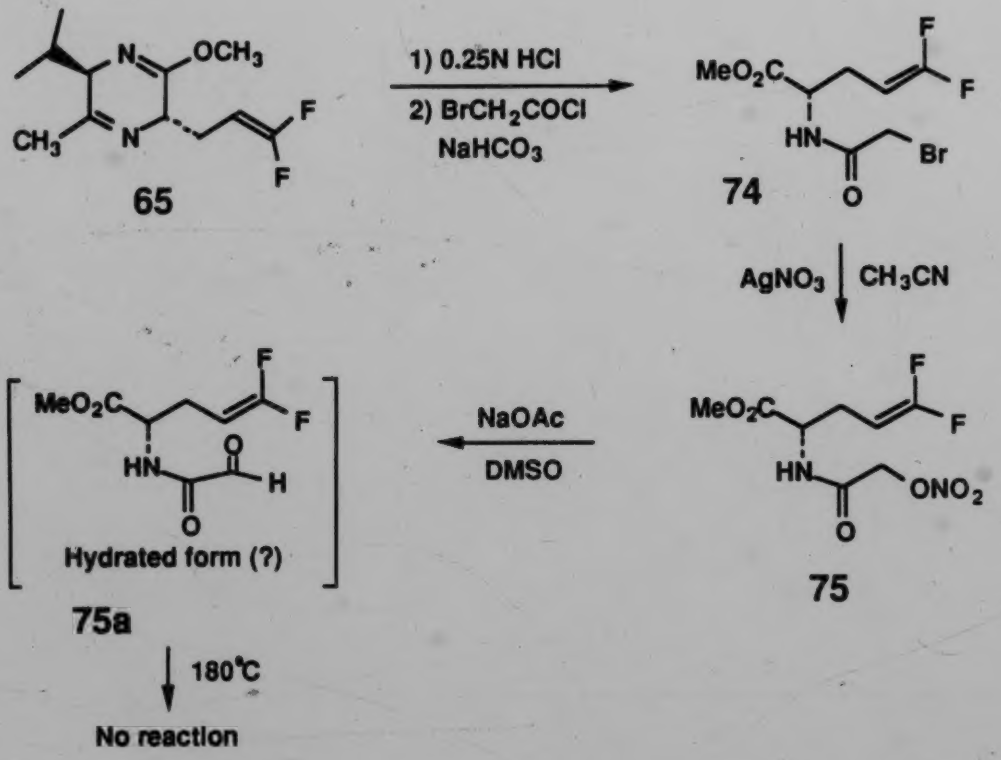
HO2C
NH2
MeOH
HCl gas
MeO2C
NH2
71
O
Cl
Br
NaHCO3
MeO2C
HN
Br
O
72
AgNO3
CH3CN
MeO2C
HN
ONO2
O
73
NaOAc
DMSO
MeO2C
HN
O
H
O
Hydrated form (?)
SnCl4
No reaction

73a

However, the reaction of **73** with DMSO in the presence of sodium acetate afforded a very complex mixture, which could not be effectively purified. A major compound of the mixture seemed to be the hydrated form of the product **73a**, but treatment with Lewis acid ($SnCl_4$) to generate the dehydrated form *in situ*, for intramolecular ene reaction gave no desired product.

In the difluoro series (Scheme 32), **65** was hydrolyzed in 0.25N aqueous HCl for 6h, and the resulting mixture was basified with $NaHCO_3$ and treated with bromoacetyl chloride. Purification by silica gel MPLC to remove the by-product, N-bromoacetyl methyl valinate, gave compound **74** in 74% yield. Subsequent reaction of **74** with silver nitrate

Scheme 32


OCH3
CH3
N
F
F
65
1) 0.25N HCl
2) BrCH2COCl
NaHCO3
MeO2C
HN
Br
O
74
AgNO3
CH3CN
ONO2
75
NaOAc
DMSO
H
Hydrated form (?)
75a
180°C
No reaction

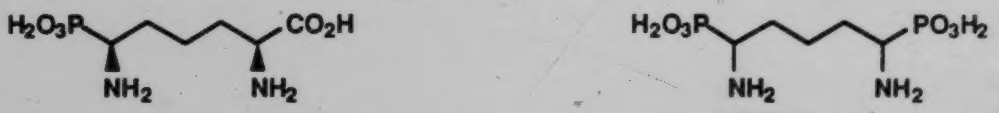
gave the nitrate **75** in 93% yield. However, a complex mixture was again obtained when **75** was treated with DMSO in the presence of sodium acetate. The mixture appeared to contain the hydrated form of the desired product **75a**, but distillation at high temperature under vacuum (180°C / 0.075 mmHg) did not generate the desired aldehyde.

In summary, approaches involving both intermolecular and intramolecular ene reactions were examined in attempts to synthesize the desired β,β-difluoro DAP isomer, but the key step could not be successfully completed.

Synthesis of Diaminopimelic Acid Phosphonate Analogues and their Dipeptides

Since phosphonate groups can often substitute for carboxyl groups and lead to enzyme inhibition as described earlier, synthesis of phosphonate analogues of diaminopimelic acid was chosen as the next target. In principle, either one or both of the carboxylic acid groups in a particular DAP isomer can be replaced by phosphonate (Figure 23). Obtaining stereochemically pure isomers of the target compounds was still a chief concern in designing synthetic approaches. Earlier synthetic work[193-208] on α-amino phosphonic acids generally employed two strategies as shown in Figure 24. One involves a nucleophilic addition of phosphite (e.g. diethylphosphite) on an imine or equivalent (e.g.

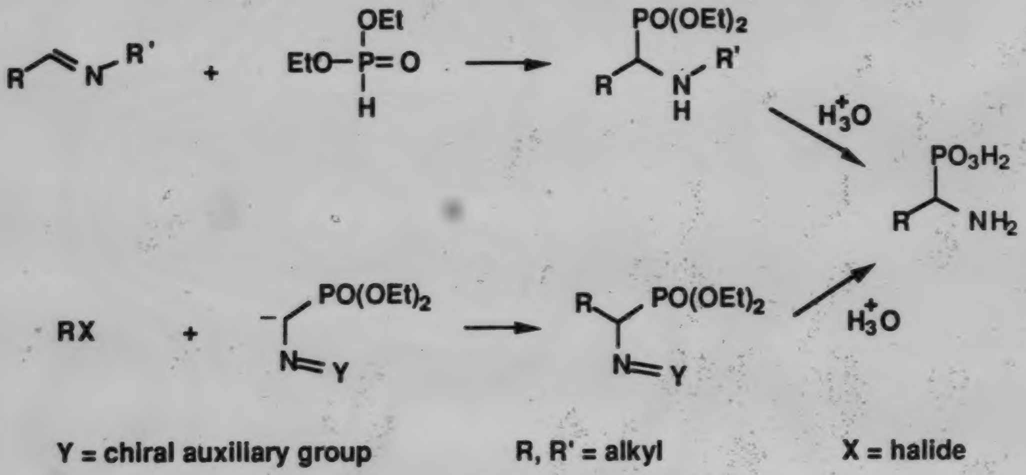
Figure 23 Target Molecules of DAP Phosphonate Analogues.



DAP monophosphonate analogue **DAP diphosphonate analogue**

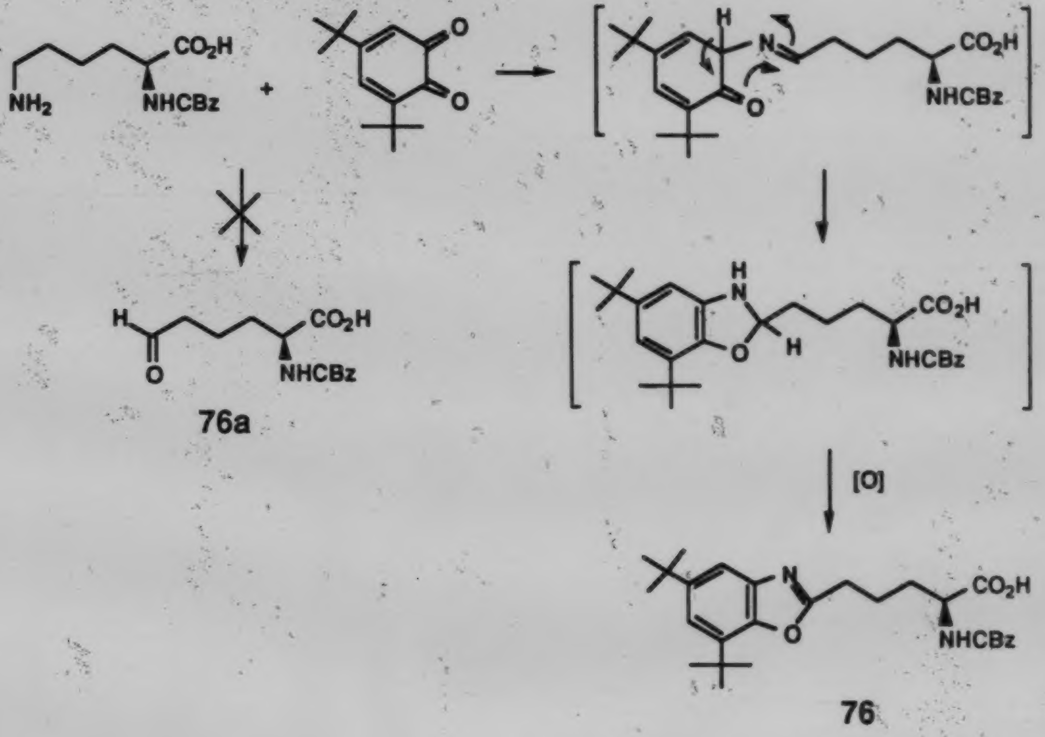
oxime). The second approach utilizes alkylation of an enolate of an aminomethylphosphonic acid (the glycine phosphonate analogue) derivative with an alkyl halide.[199-201] In this scheme, chiral auxiliary groups can be used to achieve stereocontrol.[200,201]

Figure 24 Basic Strategies for Synthesis of α-Amino Phosphonic Acids.



A rapid route to DAP monophosphonate analogues using nucleophilic addition of diethyl phosphite to an imine, requires aldehyde **76a** (Scheme 33) as the starting material. To make **76a**, the readily available L-lysine seemed an ideal starting material provided that oxidative deamination of the amino group to corresponding aldehyde was facile. There are a number of methods available for this kind of deamination.[209-211] Among them, the method developed by Corey and Achiva,[211] which employs 3, 5-di-tert-butyl o-benzoquinone as the oxidant, was chosen. However, when N^{α}-CBz L-lysine was treated with this benzoquinone, none of the desired aldehyde was obtained. Instead, the benzoxazole compound **76** was isolated in 64% yield, probably via the mechanism illustrated in Scheme 33.

Scheme 33


CO$_2$H
NH$_2$
NHCBz
+
O
O
H
N
CO$_2$H
O
NHCBz
H
CO$_2$H
O
NHCBz
76a
H
N
CO$_2$H
O
H
NHCBz
[O]
N
CO$_2$H
O
NHCBz
76

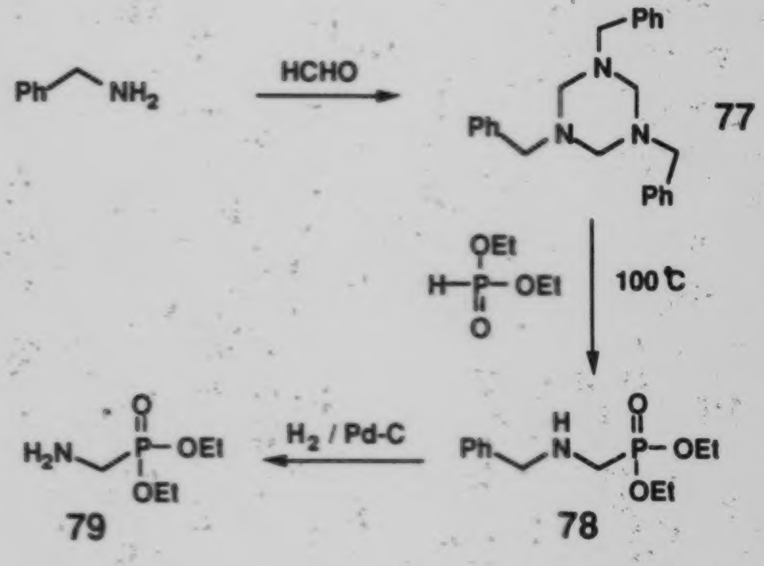
According to Rapoport and Buckley, 4-formyl-1-methylpyridinium benzenesulfonate is a convenient reagent for the conversion of primary amines to aldehydes and ketones.[209] This method mimics the biological process for transamination reactions with pyridoxal phosphate. The reagent was prepared by heating freshly distilled pyridine-4-carboxaldehyde and methyl benzenesulfonate in refluxing benzene and subsequently drying under vacuum. However, repeating the deamination reaction of N$^{\alpha}$-CBz L-lysine with this reagent following the literature procedure[209] did not produce the desired aldehyde in our hands.

Two reports appeared recently on synthesis of chiral α-aminophosphonic acids. One developed by Schoellkopf and Schutze[200] employed the imines **80** and **81**, which were derived from optically pure camphors as the chiral auxiliaries, whereas the another reported by Jacquier and colleagues[201] utilized the imine **83**, which was made from optically pure 2-hydroxy-3-pinanone. The stereoselectivity offered by these methods varied with the alkyl halides used as the alkylating reagents (d.e. 95%-11%), and in the most cases was moderate (80% - 30%). Our objective to synthesize DAP monophosphonate and diphosponate analogues in stereochemically pure form with the use of such chiral auxiliary compounds could in principle be realized either by highly stereoselective alkylation or by separation of diastereomeric products by chromatography.

Aminomethylphosphonic acid diethyl ester **79** was required in preparation of the imines. Following literature procedures[212,213] (Scheme 34), benzylamine condensed with aqueous formaldehyde to produce the triazine **77** along with a small amount of 1,3-diazetane in quantitative yield. Heating a mixture of the triazine and diethylphosphite at 100°C affords diethyl N-benzylaminomethyl phosphonate **78** (64% yield), which was hydrogenated on palladium-charcoal at normal pressure to give compound **79** in 94% yield.

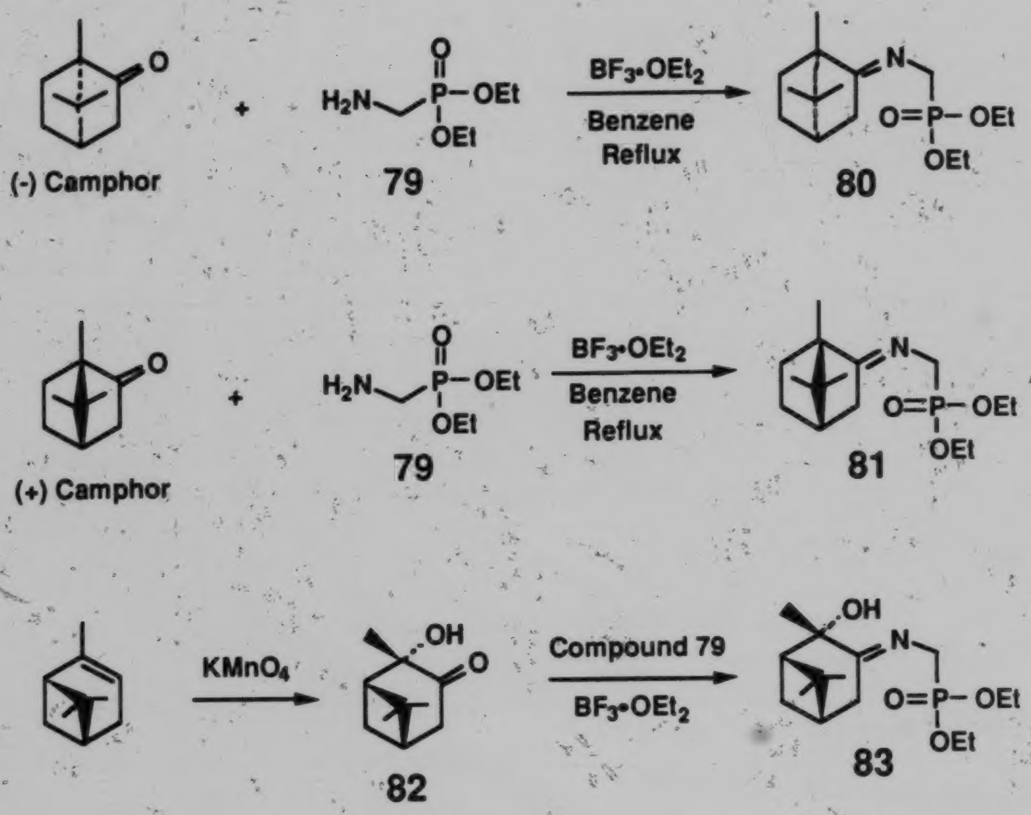
The imine formation[200] from compound **79** with (1R)-(+)-camphor (D-camphor) occurs upon heating to reflux with boron trifluoride etherate in benzene (Scheme 35). After purification by flash chromatography, **81** was distilled under vacuum (110°C / 0.01 mmHg) (38% yield) in preparation for the alkylation reaction. The enantiomer **80** could be made analogously from (S) camphor on **79** in 41% yield.

Scheme 34



An alternative chiral auxiliary, 2-hydroxy-3-pinanone (**82**) was made by the oxidation of commercially available (+) α-pinene by potassium permanganate in 40% yield (Scheme 35).[214] After distillation under vacuum (115°C / 17 mmHg), the ketol **82** was similarly treated with aminomethyl phosphonate **79** to form the corresponding imine **83**. The imine was purified by flash chromatography (45% yield), but attempted distillation (145°C / 0.05 mmHg) caused partial decomposition.

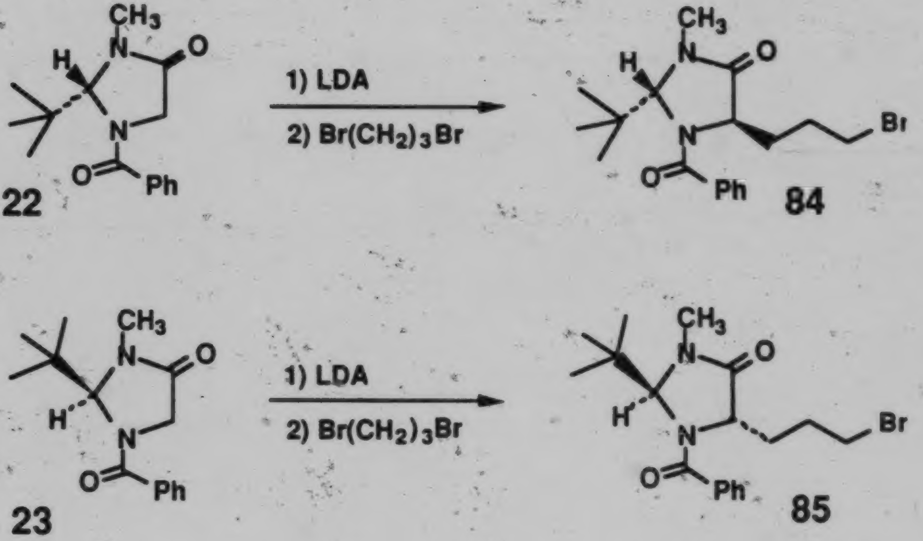
Scheme 35



O
(-) Camphor
+
H₂N
P—OEt
OEt
79
BF₃•OEt₂
Benzene
Reflux
N
O=P—OEt
OEt
80
(+) Camphor
81
KMnO₄
OH
82
Compound 79
83

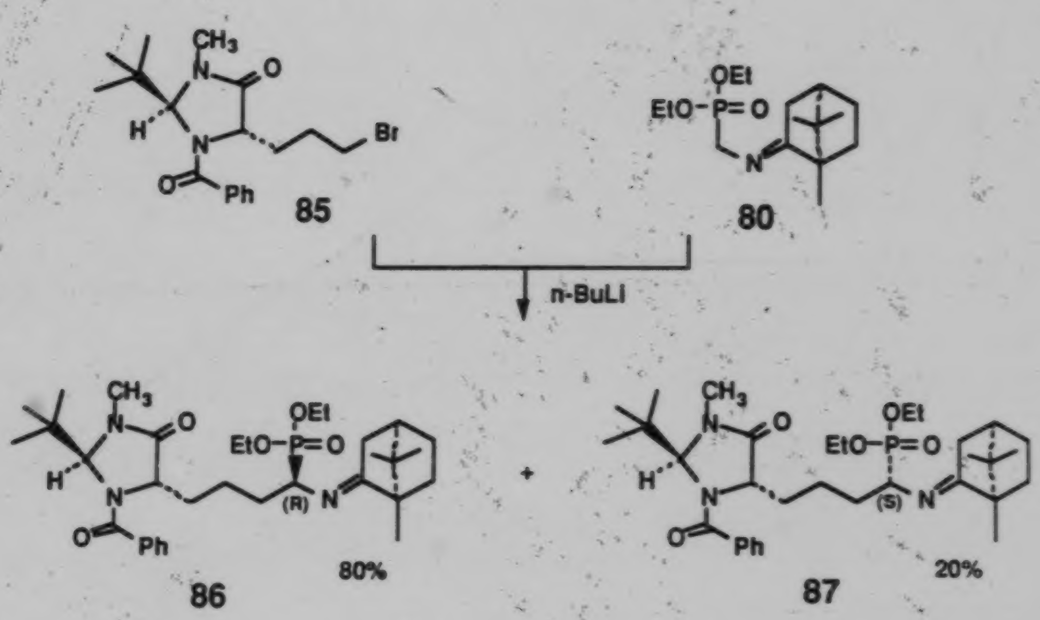
With these chiral imines available, the next goal was synthesis of the required alkylating reagents. For this purpose, the Seebach imidazolidinone **22** was alkylated with 1.2 equivalent of 1, 3-dibromopropane (Scheme 36) to give the monobromide **84** (66% yield). The diastereoselectivity for the alkylation is very high. None of the other diastereomer could be detected in either ^{1}H NMR or ^{13}C NMR spectra. Its enantiomer **85** was similarly synthesized from imidazolidinone **23** in 57% yield. Alkylation of the enolate of imine **80**, generated by n-BuLi, with monobromide **85** (Scheme 37) gave, after purification by flash chromatography, two diastereomers **86** and **87** in a ratio of 4 : 1 as a mixture (71% yield).

Scheme 36



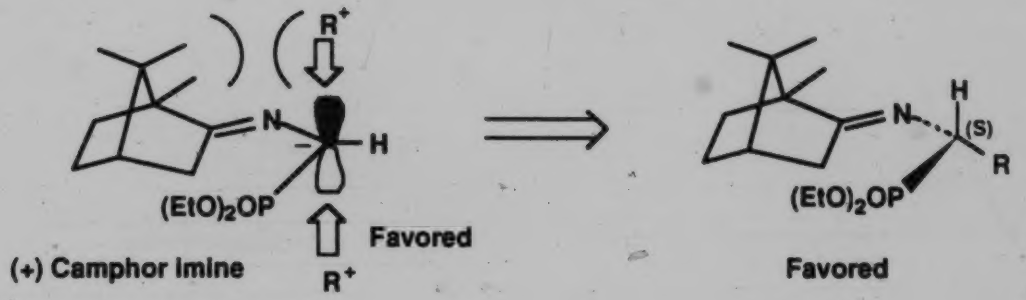
The two isomers were distinguishable on ^{1}H NMR and ^{31}P NMR spectra. Experimentally, the reaction temperature during the alkylation was gradually raised from an initial -78°C to room temperature. Since monobromide **85** is not a reactive reagent compared with allyl or benzyl bromide, the alkylation is believed to actually occur at a relatively high temperature. Since literature precedent[200,201] suggests that lower reaction temperature tends to give higher stereoselectivity, a less reactive alkylating reagent such as **85** tended to produce more of the minor isomer (i.e. **87**). According to the original work,[200] alkylation of the imine **80** derived from (-) camphor preferentially generates an isomer with (R) configuration at the

Scheme 37



newly created chiral center, whereas the imine **81** from (+) camphor selectively produced the isomer of (S) configuration. This can be rationalized as shown in Figure 25. Upon enolization of the imine by n-BuLi, the enolate may adopt the conformation shown to avoid a severe interaction with the bridgehead methyl group. Assuming the orbital of the anion is parallel with the imine π-bond to achieve a resonance stabilization, an incoming alkyl halide would preferentially approach the anion from the less hindered side (bottom) to avoid interactions with the dimethyl group, thus producing (S) configuration at the newly created chiral center. Based on the well-precedented stereoselectivity of the camphor imines,[200] the configuration of the newly generated chiral center in the major isomer **86** was assigned as (R), and the one in the minor isomer **87** was assigned as (S).

Figure 25 Possible Interaction of the Enolate of Camphor Imine **81** with Electrophiles.

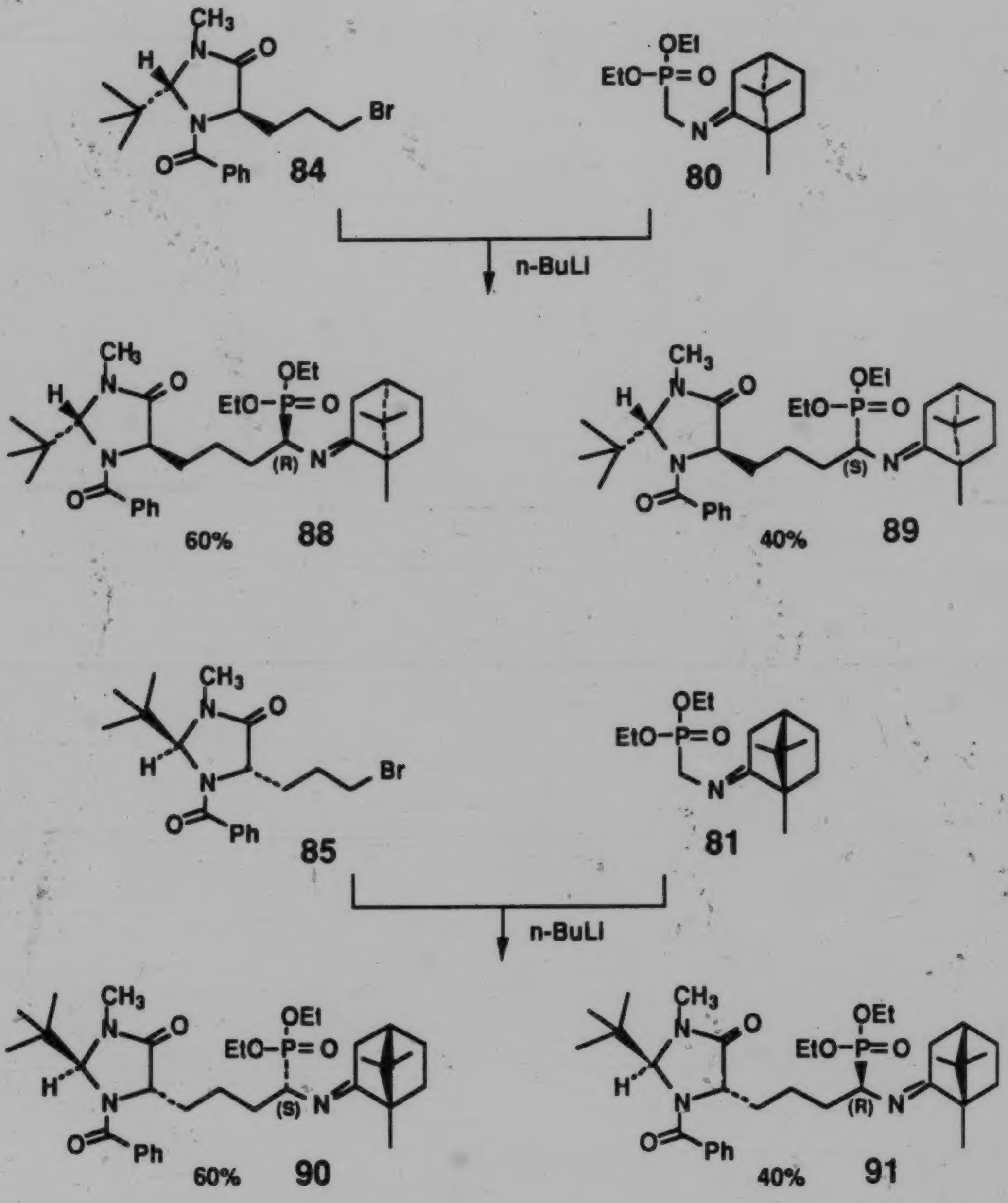


When the same imine **80** was alkylated with another bromide **84** (Scheme 38), two diastereomers **88** and **89** in a ratio of 3 : 2 were isolated in 86% yield. These two isomers were also distinguishable by their ^{1}H NMR and ^{31}P NMR spectra and their stereochemistry was analogously assigned. As expected, the alkylation of imine **81** (the enantiomer of imine **80**) with bromide **85** (the enantiomer of bromide **84**) produced two diastereomers **90** and **91** in the same ratio (3 : 2). Since the two products **90** and **91** are enantiomers of isomers **88** and **89**, respectively, the two enantiomeric pairs (compounds **90** and **88**, compounds **91** and **89**) have identical spectroscopic properties.

Literature reports[200,201] appeared to suggest that alkylation of 2-hydroxy-3-pinanone imine **83** with non-reactive alkyl halides gives higher diastereoselectivity than the camphor imine **80** or **81**. Nevertheless, the alkylation of imine **83** with bromide **84** produces none of the desired alkylation product.

Since diastereoselectivities of the two alkylation reactions were modest, the best means to obtain the DAP monophosphonate isomers in stereochemically pure form was separation of these diastereomers by chromatography. Fortunately, silica gel MPLC column can separate isomer **86** from **87** and isomer **88** from **89**, although the resolution is

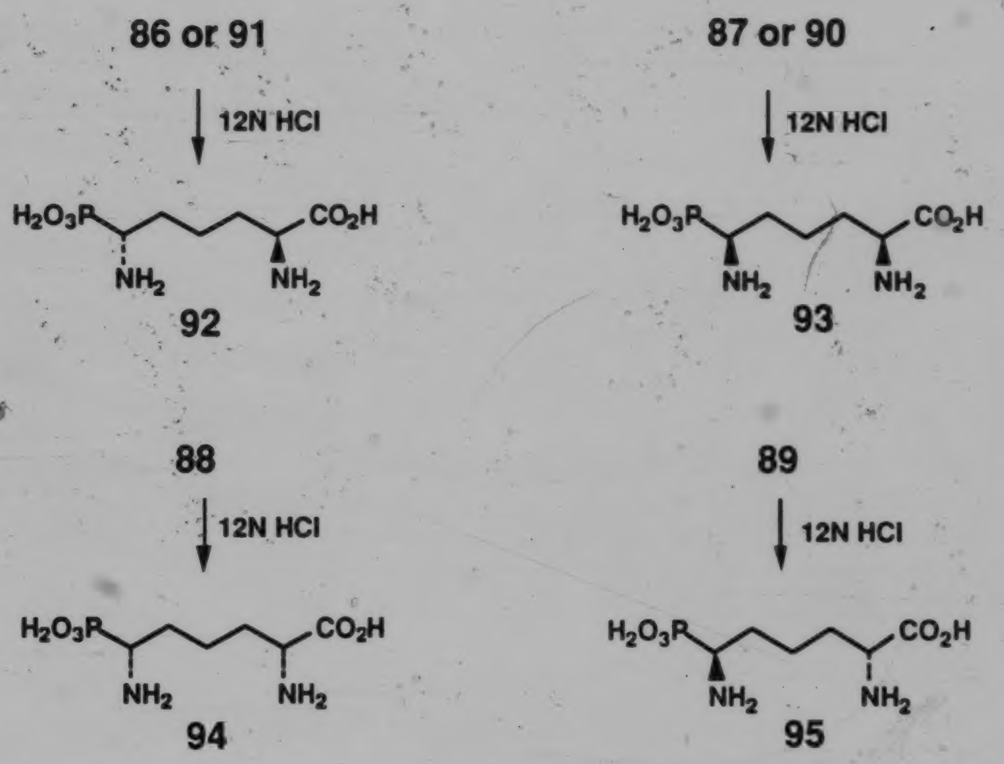
Scheme 38


84
80
n-BuLi
(R)
60%
88
(S)
40%
89
85
81
n-BuLi
(S)
60%
90
(R)
40%
91

difficult. The major isomers **86** and **88** were obtained in pure form, and their ^{1}H NMR and ^{31}P NMR spectra showed none of the corresponding minor isomers. The minor isomers **87** and **89** were also separated in relatively pure form such that their ^{1}H NMR and ^{31}P NMR spectra indicated less than 5% of the corresponding major isomer as a contaminant.

Although hydrolysis of the compounds **86**, **87**, **88** and **89** by directly heating to reflux in 12N HCl proved problematic, two stage cleavage of the compounds in 12N HCl at room temperature for 1 day, followed by heating to reflux for 3 days was an ideal procedure to generate the free monophosphonate DAP isomers (Scheme 39).

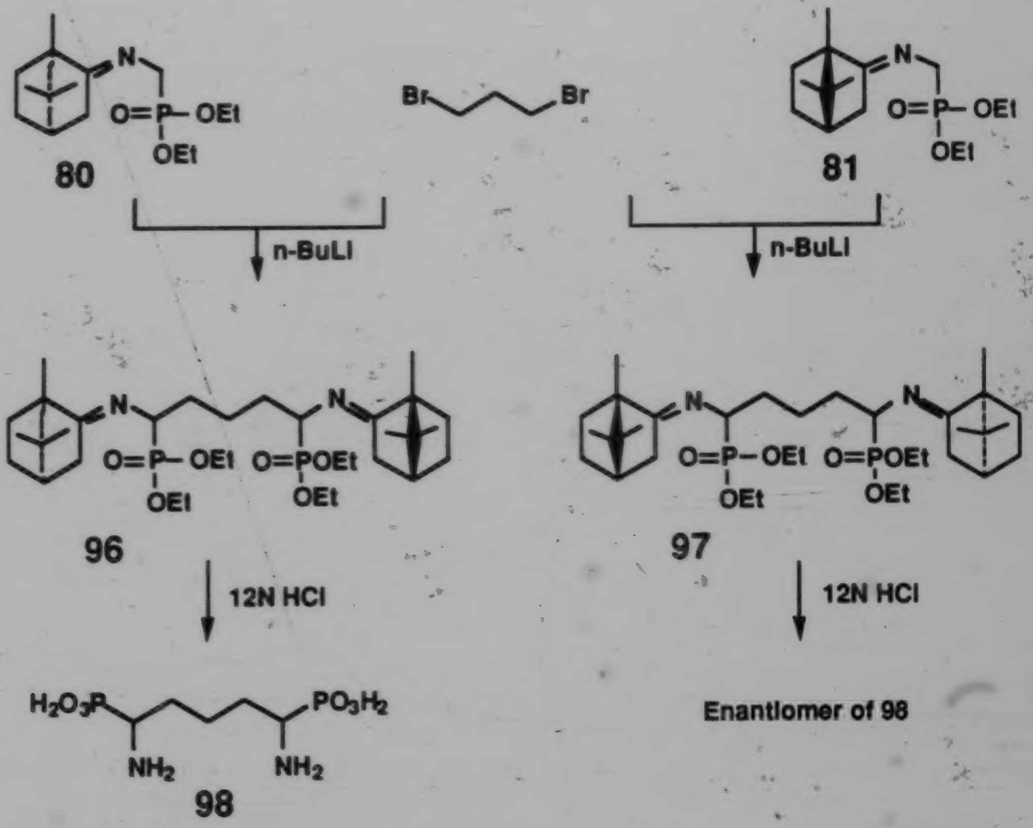
Scheme 39


86 or 91
12N HCl
H_2O_3P
CO_2H
NH_2
NH_2
92
87 or 90
12N HCl
H_2O_3P
CO_2H
NH_2
NH_2
93
88
12N HCl
H_2O_3P
CO_2H
NH_2
NH_2
94
89
12N HCl
H_2O_3P
CO_2H
NH_2
NH_2
95

Although crystallization with propene epoxide has been utilized to purify phosphonate amino acid analogues, the phosphonate DAP isomers **92**, **93**, **94** and **95** were best purified by cellulose chromatography.

To synthesize the diphosphonate DAP analogue **98** (Scheme 40), alkylation of the chiral imine **80** was done with 0.5 equivalent 1, 3-dibromopropane such that the dibromide would bridge two molecules of the imine. The protected diphosphonate **96** was isolated as a mixture of diastereomers in 62% yield after purification by flash chromatography.

Scheme 40

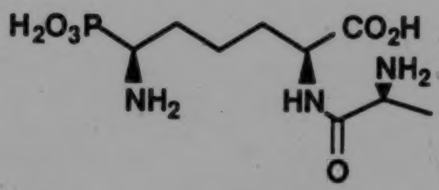


N
O=P–OEt
OEt
80
Br
Br
81
n-BuLi
n-BuLi
96
97
12N HCl
12N HCl
H_2O_3P
PO_3H_2
NH_2
NH_2
98
Enantiomer of 98

Since the process involves two consecutive alkylations and generation of two new chiral centers, four possible diastereomers form. These were not separated, but were hydrolyzed directly to the DAP diphosphonate analogue **98** (63% yield). A similar alkylation of imine **81** by 1, 3-dibromopropane gives **97** (65% yield), which upon hydrolysis in 12N HCl affords the diphosphonate **98** as a mixture of diastereomers. The two diphosphonate mixtures generated above are enantiomeric to each other and have identical physical properties.

After completion of these syntheses for enzyme inhibition studies, the dipeptide derivatives of the DAP monophosphonates appeared to be attractive targets that could improve transport into bacterial cells, and thus antibiotic activity. The initial dipeptide chosen was L-alanyl-DAP phosphonate, whose structure is shown in Figure 26.

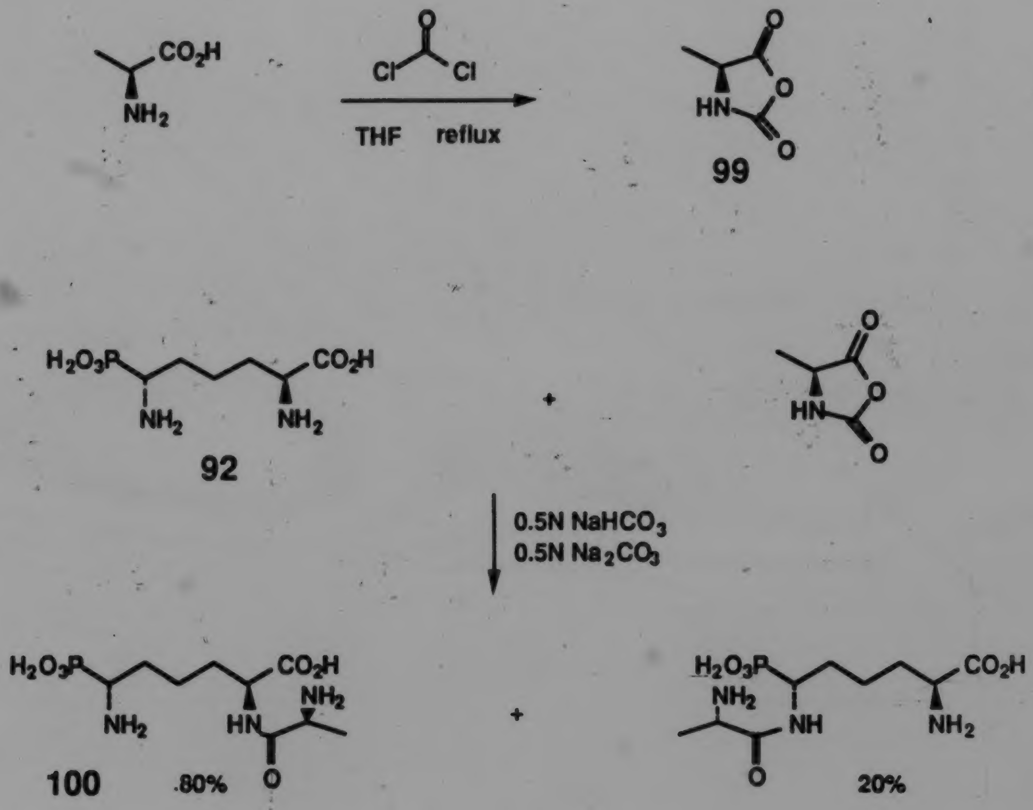
Figure 26 A DAP Phosphonate Dipeptide with L-Alanine.



Usually peptide synthesis involves a series of protection and deprotection procedures, and since there are two amino groups in DAP phosphonate, selective protection would normally be required. However, the two amino groups are differentiated by their proximity to the carboxylate group and the phosphonate function. Phosphonic acids are more acidic than corresponding carboxylic acids, and form divalent anions whereas the carboxylate exists as a monovalent anion. In addition, the size of the phosphonate group is large compared to the carboxylate. Therefore there seemed to be a reasonable chance to selectively form a peptide bond on the amino group at one of the two ends.

To synthesize the dipeptide with L-alanine (Scheme 41), DAP monophosphonate **92** was dissolved in a buffer of 0.5N $NaHCO_3$ / 0.5N Na_2CO_3 and treated with L-alanine N-carboxyanhydride (**99**) in DMF. Compound **99** was available in 54% yield by treatment of L-alanine with phosgene (Scheme 41) followed by reflux in THF. A difficult separation of the dipeptide mixture by cellulose chromatography afforded compound **100** and the regioisomer in 80 : 20 ratio (42% overall yield) as well as recovered **92** (49%). ^{1}H NMR

Scheme 41



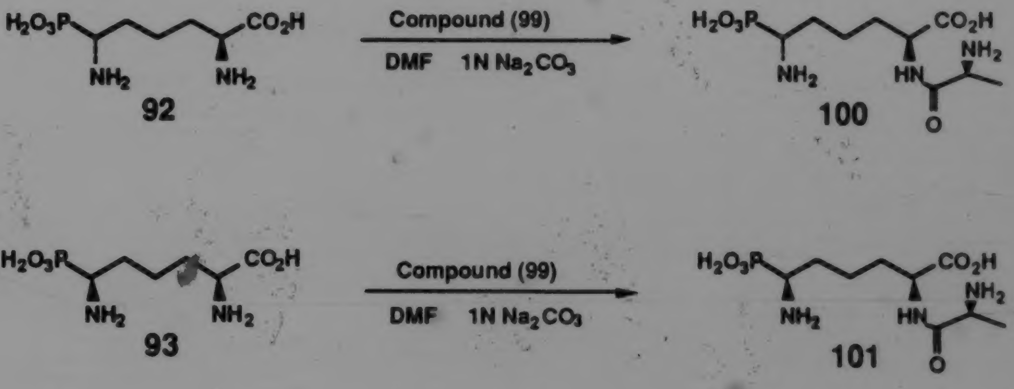
CO2H
NH2
O
Cl
Cl
THF reflux
O
O
HN
O
99
H2O3P
CO2H
NH2
NH2
92
+
O
O
HN
O
0.5N NaHCO3
0.5N Na2CO3
H2O3P
CO2H
NH2
NH2
HN
O
100
80%
+
H2O3P
CO2H
NH2
NH
NH2
O
20%

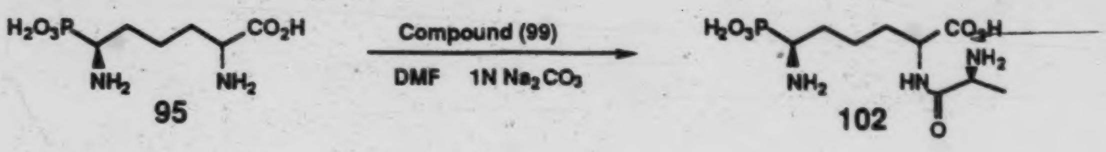
spectroscopy could be utilized to determine which peptide bond was formed. Upon acylation of an amino group, the signal for the α-proton adjacent to it shifts downfield by 0.3 - 0.4 ppm. The results show that under these reaction conditions the two amino groups can not be sufficiently differentiated to permit a completely selective peptidation.

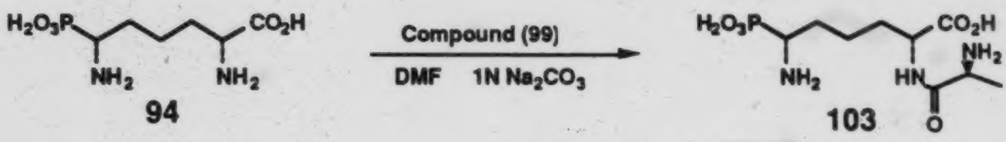
However, a buffer of 1N aqueous Na_2CO_3, which has a higher pH value than the 0.5N $NaHCO_3$ /0.5N Na_2CO_3 (pH 10) buffer used previously, reduces production of the unwanted minor peptide such that its presence can hardly be detected by ^{1}H NMR spectroscopy of the reaction mixture. Despite the low yield associated with this peptidation (47% - 19%), the desired dipeptide **100** could be isolated in pure form (Scheme 42).Dependance of the selectivity on the pH of the reaction medium may be due to the fact that at higher pH, the phosphonic acid group exists as a divalent anion instead of the monovalent anion. The divalent anion may be more solvated in the aqueous solution than the monovalent species, thereby making the peptidation at phosphonate end more difficult.

The peptidations of other DAP monophosphonate isomers **93**, **95** and **94** with alanine N-carboxy anhydride (**99**) proceed similarly in 1N Na_2CO_3 buffer to afford the corresponding dipeptides **101** (23% yield), **102** (19% yield) and **103** (19% yield), respectively (Scheme 42).

Scheme 42




H2O3P
CO2H
NH2
NH2
95
Compound (99)
DMF 1N Na2CO3
H2O3P
CO2H
NH2
NH2
HN
O
102


H2O3P
CO2H
NH2
NH2
94
Compound (99)
DMF 1N Na2CO3
H2O3P
CO2H
NH2
NH2
HN
O
103

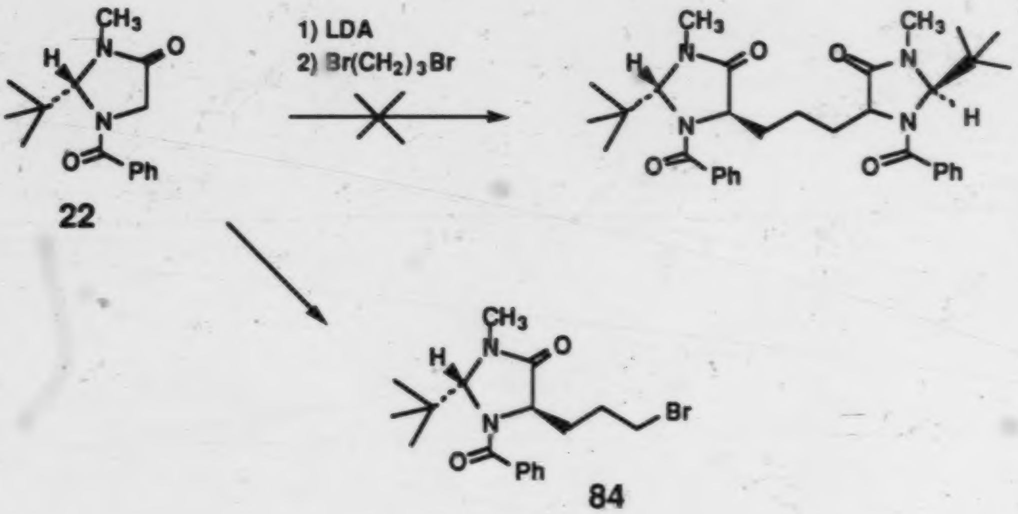
In summary, four DAP monophosphonate isomers **92**, **93**, **94** and **95** were synthesized in stereochemically pure form by alkylation of the camphor imine **80** or **81** with bromide **84** or **85**, followed by separation of the diastereomers thus formed. Furthermore, four dipeptides **100**, **101**, **102**, and **103** of these DAP monophosphonates were prepared by selective reaction of the isomers with L-alanine N-carboxyanhydride **99**. The DAP diphosphonate analogue was also successfully made as a mixture of four possible isomers by the alkylation of camphor imine **80** or **81** with 1, 3-dibromopropane.

Stereocontrolled Synthesis of Diaminopimelic Acid Isomers and Their Selectively Protected Derivatives

Enzyme inhibition studies require pure DAP isomers and selectively protected DAP is essential for synthesis of immunoadjuvants and peptidoglycan analogues. Although pure DAP isomers are available by multistep classical resolution procedures, an efficient stereospecific synthesis of pure DAP isomers and their selectively protected derivatives is highly desirable.

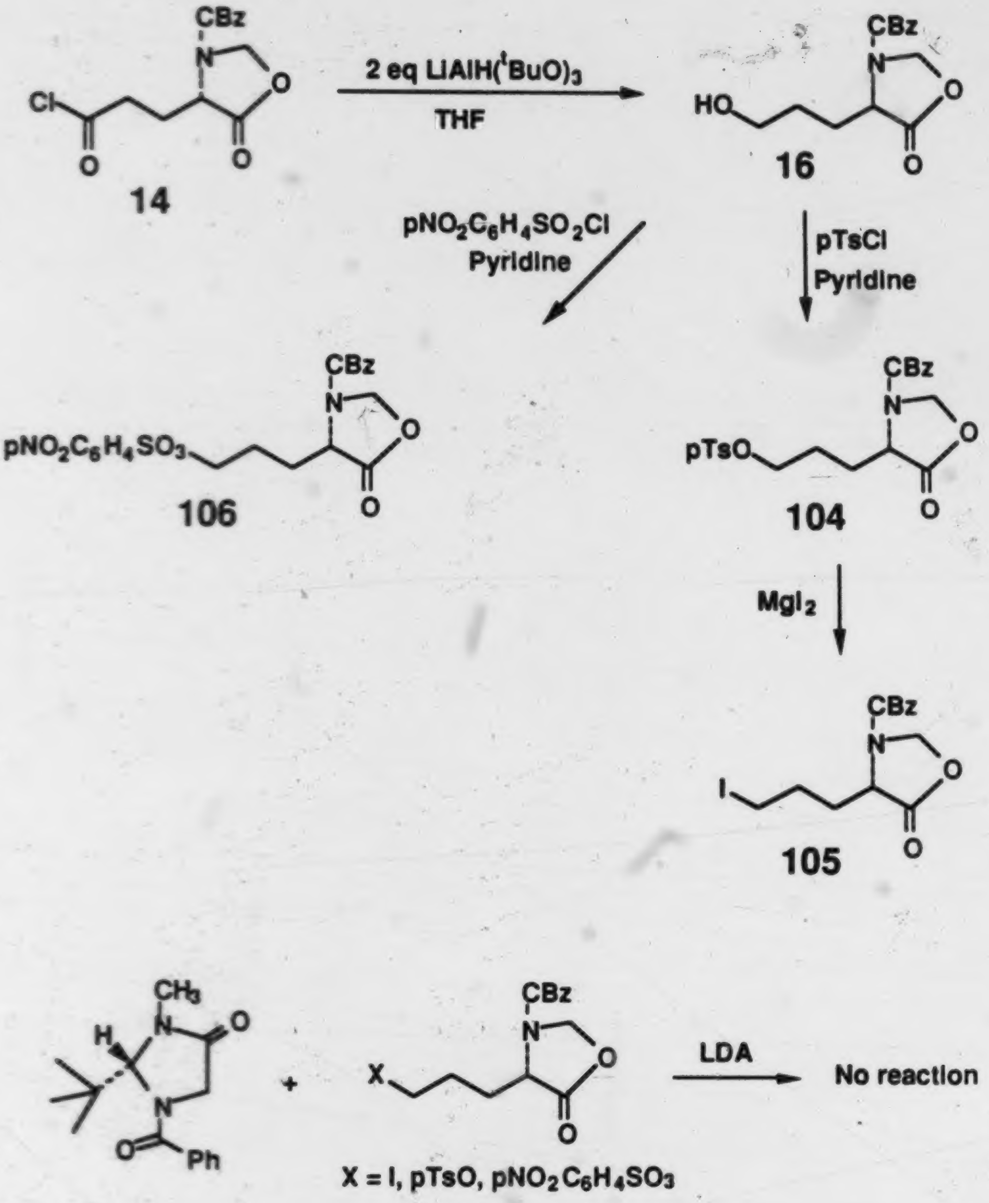
Seebach's chiral imidazolidinone **22** or **23** can induce high diastereoselectivity in alkylations with various halides, even in a case where the halide is not activated.[178-180] In an initial approach, the enolate of the imidazolidinone **22** was treated with 0.5 equivalent of 1, 3-dibromopropane in an attempt to bridge two molecules of **22** (Scheme 43) in a fashion analogous to synthesis of the diphosphonates **96** and **97** (Scheme 40). However, no desired product was obtained and instead the monobromide **84** was formed in 22% yield. This suggests that the double alkylation of **22** with bromide **84** is not feasible, and an alternative approach is necessary.

Scheme 43



Initial studies explored use of different alkylating agents for reaction with the enolate of **22**. To generate these, acyl chloride **14** (derived from L-glutamic acid) was treated with 2 equivalents of LiAlH(^{t}BuO)$_3$, to produce alcohol **16** in 78% yield (Scheme 44). Three alkylating agents were synthesized from **16**. These include the toluenesulfonate **104**, the iodide **105**, and the p-nitrobenzenesulfonate **106**. The alcohol **16** reacts with toluenesulfonyl chloride in the presence of pyridine to form the tosylate **104** in 83% yield. The iodide **105** was generated from the tosylate **104** by the action of magnesium iodide in

Scheme 44


CBz
Cl
O
O
O
N
14
2 eq LiAlH(ᵗBuO)₃
THF
HO
16
pNO₂C₆H₄SO₂Cl
Pyridine
pTsCl
Pyridine
pNO₂C₆H₄SO₃
106
pTsO
104
MgI₂
I
105
CH₃
H
Ph
+
X
LDA
No reaction
X = I, pTsO, pNO₂C₆H₄SO₃

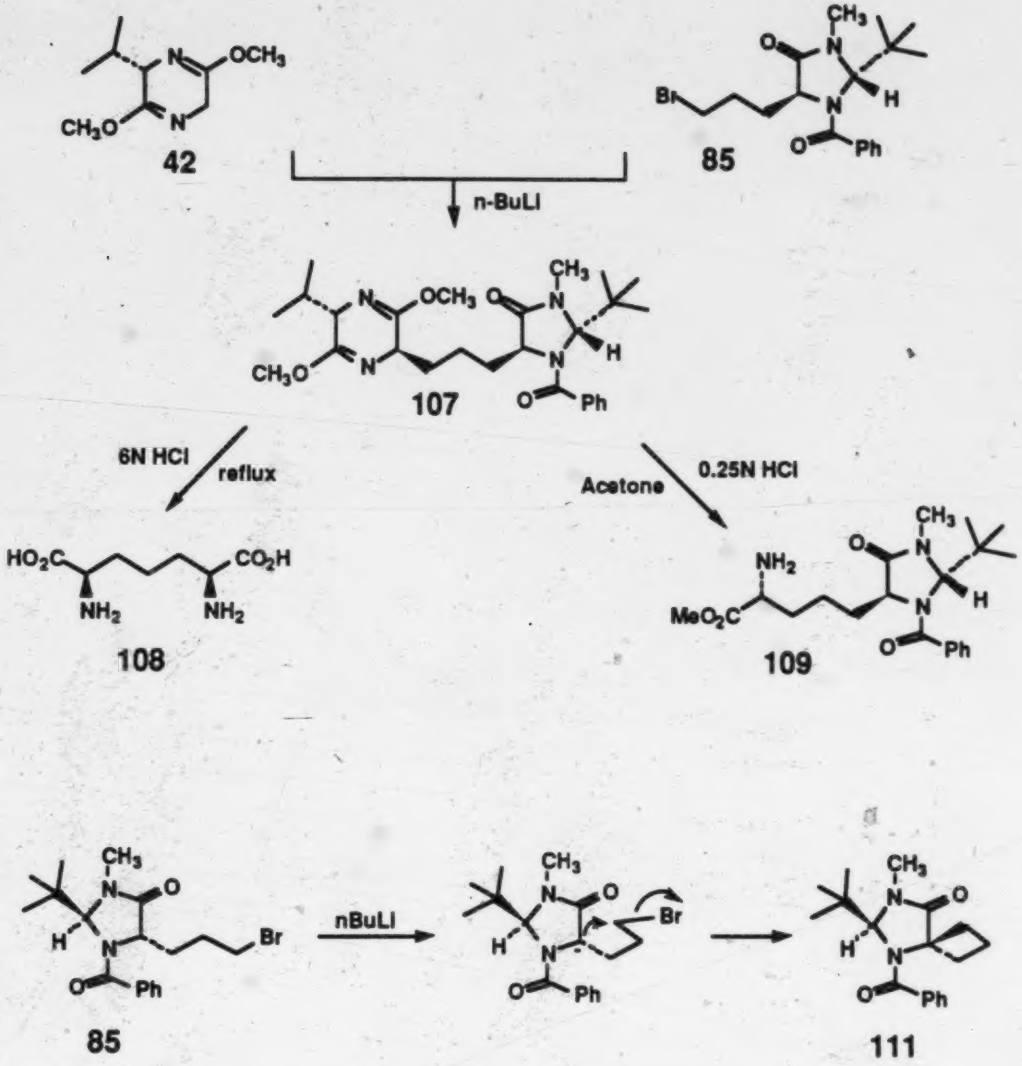
50% yield. Treatment of the alcohol **16** with p-nitrobenzenesulfonyl chloride gives the p-nitrobenzenesulfonate **106** in 49% yield. Unfortunately, attempted alkylations of the enolate of imidazolidinone **22** with these compounds failed to generate any of the desired products. At this stage, it appeared that the Seebach imidazolidinone was not an appropriate chiral auxiliary compound for such reactions.

The Schoellkopf bislactim ether **42** was therefore chosen for further alkylation reactions. When the lithium enolate of **42** was treated with the bromide **85** (Scheme 45), the expected alkylation occurred to give the desired product **107** in 30% yield. A side product identified as **111** arises from an intramolecular alkylation in 5% yield. On the basis of ^{1}H NMR and ^{13}C NMR analysis, **107** exhibits high diastereomeric purity (>90% d.e.). This may be due to the high diastereoselectivity of the alkylation or to chromatographic separation from possible diastereomers. Subsequent hydrolysis of **107** in refluxing 6N HCl followed by purification by cellulose chromatography affords the expected *meso* isomer of DAP **108** in 70% yield.

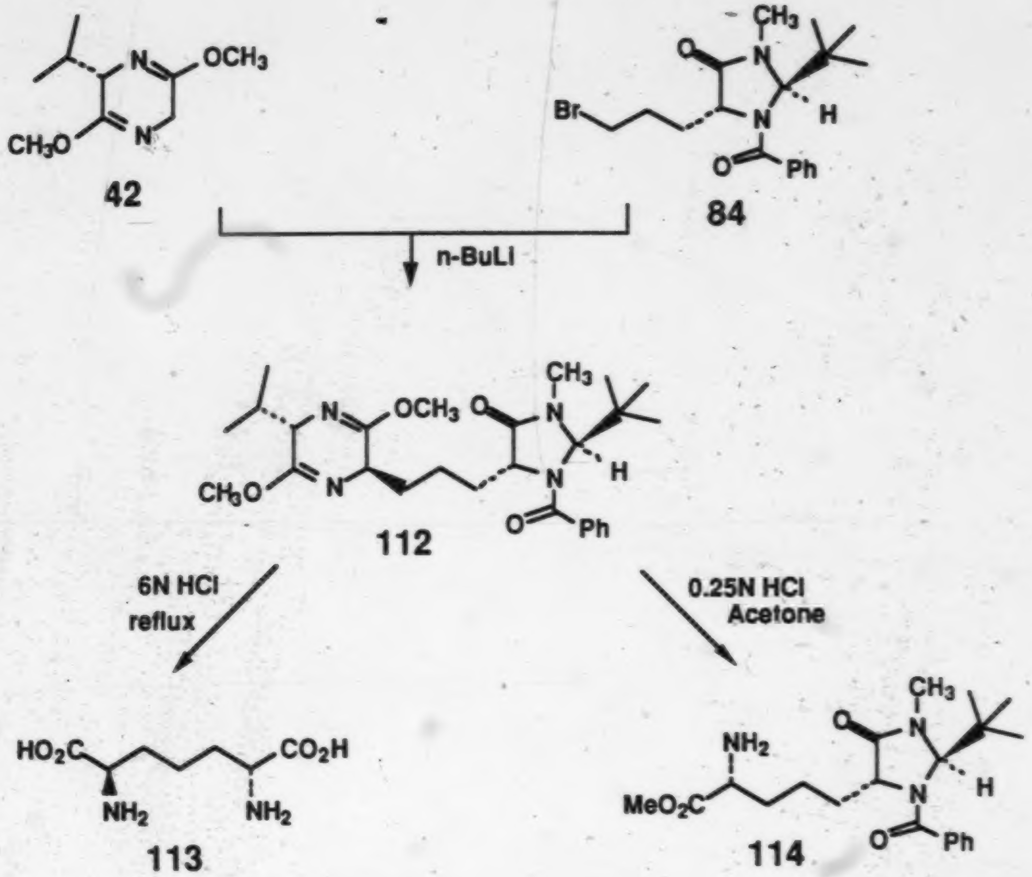
Although full deprotection of **107** was one target of this synthesis, another was to partially unmask **107** to selectively protected DAP derivative suitable for further elaboration. Since the bislactim ether ring is much more susceptible to hydrolysis than the imidazolidinone ring,[162,178] mild conditions could selectively deprotect **107** at the bislactim side. Hydrolysis of **107** in 0.25N aqueous HCl and acetone at room temperature gives the partially unmasked **109** in 85% yield.

Similarly, alkylation of the bislactim ether **42** with bromide **84** generates **112** in 30% yield (Scheme 46). Similar hydrolysis conditions allow formation of either fully deprotected L,L-DAP **113** (68% yield) or of partially deprotected amino ester **114** (86% yield).

Scheme 45


42
85
n-BuLi
107
6N HCl
reflux
Acetone
0.25N HCl
108
109
85
nBuLi
111

Scheme 46



OCH3
CH3O
42
CH3
O
N
N
H
Br
O
Ph
84
n-BuLi
112
6N HCl
reflux
0.25N HCl
Acetone
HO2C
CO2H
NH2
NH2
113
NH2
MeO2C
114

In summary, a new synthetic approach has been devised for the synthesis of pure stereoisomers of DAP and their selectively deprotected derivatives. The synthesis involves alkylation of the Schoellkopf bislactim ether **42** with bromide **84** or **85**, which leads to **107** or **112**. Hydrolysis of these compounds under controlled conditions gives either the DAP isomers or their partially deprotected forms.

Biological Activities of DAP Analogues

(1) Inhibition of DAP Epimerase by Fluorinated DAP Isomers

(The enzymology experiments on DAP epimerase with fluorinated DAP isomers were done in collaboration with Professor Michael H. Gelb and Yukang Lin in the Department of Chemistry, University of Washington. These studies have been published,[97] and the following description is excerpted from this manuscript.)

The two β-hydroxy DAPs **26** and **29** were tested as inhibitors of DAP epimerase. The epimerase was assayed by following the release of tritium into the solvent from

Table 3 Interaction of β-Substituted DAP Analogues with DAP Epimerase

Compound	IC_{50}, μM[a]	k_{cat} for HF loss,[b] s^{-1}
26	4000	0
29	2500	0
54	4	0.049
55	10	5.22
56	25	2.35
57	8	0.049

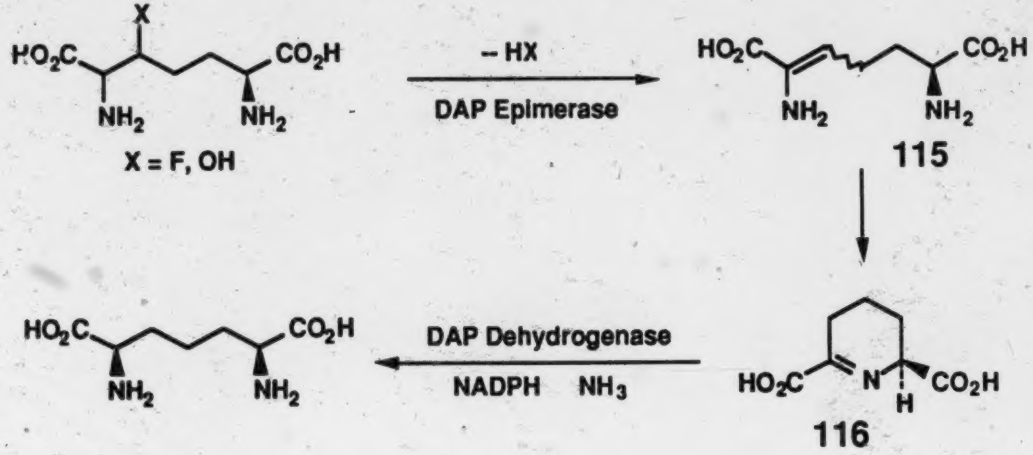
[a] Determined by the radiometric assay. [b] The k_{cat} value for the DAP epimerase catalyzed epimerization of L,L-DAP to *meso*-DAP is 84 s^{-1}.

uniformly tritiated DAP. Compounds **26** and **29** were found to be weak inhibitors of DAP epimerase, producing 50% inhibition at concentrations of 4.0 and 2.5 mM, respectively.

However, the corresponding β-fluoro DAP analogues **54**, **55**, **56** and **57** were found to be potent inhibitors of DAP epimerase. By radiometric assay, **54**, **55**, **56** and **57** produced 50% inhibition at concentrations of 4, 10, 25 and 8 μM, respectively (Table 3). These IC_{50} values are well below the K_m values of 320 μM and 160 μM for *meso*-DAP and L,L-DAP, respectively.

Experiments were undertaken to determine whether DAP-epimerase would catalyze the elimination of water from **29** and **26** to produce an enamine **115** which would spontaneously cyclize (Scheme 47) to tetrahydrodipicolinic acid **116**. Compound **116** is an intermediate in the lysine / DAP pathway in bacteria (Figure 3) and is also a substrate for the enzyme *meso*-DAP dehydrogenase which catalyzes the reductive amination of **116** to

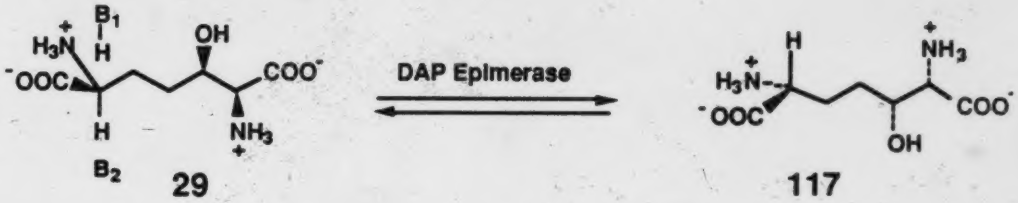
Scheme 47


X
HO2C
CO2H
NH2
NH2
X = F, OH
– HX
DAP Epimerase
HO2C
CO2H
NH2
NH2
115
HO2C
N
H
CO2H
116
DAP Dehydrogenase
NADPH NH3
HO2C
CO2H
NH2
NH2

produce *meso*-DAP. Thus, the production of **116** can be followed in the presence of DAP-dehydrogenase by monitoring the decrease in absorbance at 340 nm as NADPH is consumed. By using this method, it was seen that analogs **29** and **26** were not converted to **116**.

The epimerization of **29** and **26** by DAP-epimerase was also studied. Epimerization of compound **26**, which is an analog of *meso*-DAP, is forced to occur at the hydroxylated end of the molecule. This is based on the fact that the region of the enzyme which binds the non-epimerized end of DAP can only accommodate the L-configuration.[47] No epimerization product was detected when compound **26** was incubated with DAP-epimerase. Compound **29** is an analog of LL-DAP and its interaction with DAP-epimerase is more complicated in that epimerization could, in principle, occur at either end of the molecule. However HPLC analysis and deuterium exchange experiments indicated that the epimerization of **29** occurred primarily at the non-hydroxylated end of the molecule (Scheme 48).

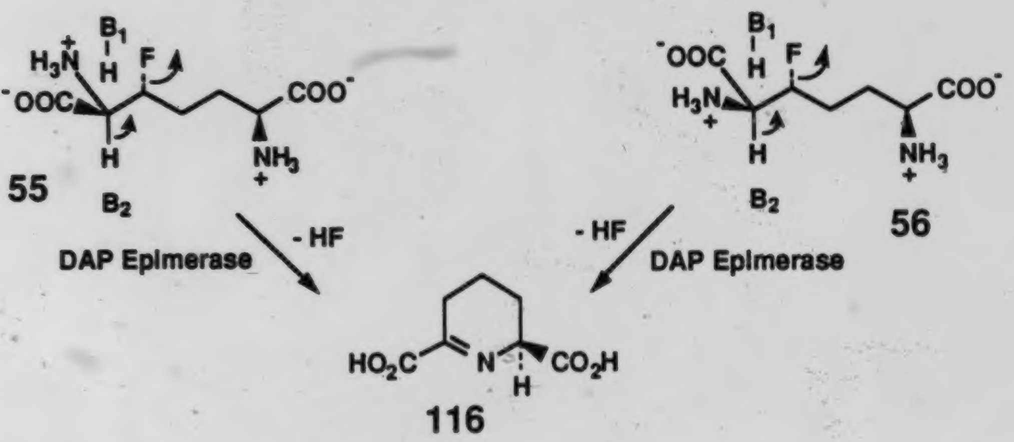
Scheme 48



As described above, enzyme-catalyzed elimination was not seen with the hydroxy DAP analogs **29** and **26**. Although this might initially be attributed to the poorer leaving group potential of the hydroxyl group compared to the fluoro group, a more significant feature is that addition of a hydroxyl group to the DAP skeleton can prevent binding of the analog to the enzyme in a catalytically productive manner. For example, the analog of *meso*-DAP, compound **26**, which in principle could be epimerized on the hydroxylated end, was not a substrate for DAP-epimerase. In addition the corresponding derivative of L,L-DAP, **29** was epimerized exclusively at the non-hydroxylated end even though epimerization at the hydroxylated end is conceivable.

It is possible that DAP-epimerase catalyzes the elimination of HF from the fluoro DAP analogs to produce imine **116**. Indeed, the formation of imine **116** from compound **56** was observed (Scheme 49). DAP epimerase-catalyzed HF elimination was also detected with isomer **55**. In contrast, studies indicated that DAP-epimerase catalyzed a much slower release of fluoride from isomers **54** and **57**. The maximal velocities for the conversion of the fluoro DAP analogs to **116** are given in Table 3. The removal of HF from the **54 / 57** mixture occurred with an overall V_{max} that was 0.058% of the V_{max} for epimerization of L,L-DAP to *meso*-DAP.

Scheme 49

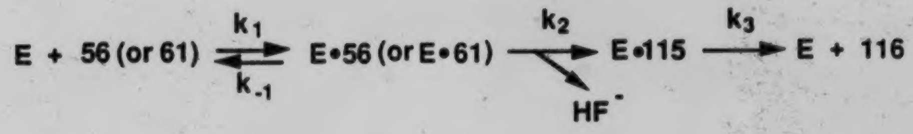


It is clear that the fluorinated DAP analogs are better classified as slow competitive substrates for DAP-epimerase rather than as simple competitive inhibitors. The enzyme-bound intermediate **115** (Scheme 47) could potentially suffer attack by a nucleophilic functionality on the enzyme, such as the active site thiol, to form a covalently-bound enzyme-inhibitor adduct. Since none of the fluoro DAP analogs were irreversible inhibitors of the epimerase, the formation of such an adduct would have to be a reversible process.

Since the fluorinated DAP analogs are actually consumed by DAP-epimerase, it might be expected that the inhibitory properties of these compounds would diminish if they are preincubated with the enzyme prior to inhibition tests. This was shown to be the case. When compound **56** was preincubated with the epimerase, the degree of inhibition continuously decreased to zero over 20 min. In contrast, the inhibition by **54**, which is consumed much more slowly than **56**, did not change over this same period. Thus **54** is not only a more potent inhibitor than **56**, it is also the more persistent inhibitor.

With the analog of **56** deuterated at C-2, compound **61**, the isotope effects on the kinetic parameters for the DAP-epimerase catalyzed elimination of HF were determined. An isotope effect on V_{max} of 1.1 and on K_m of 0.58 was observed. In light of the results, a kinetic scheme (Figure 27) for the interaction of **56** with the epimerase is shown below.

Figure 27 Kinetic Scheme for the Interaction of **56** (or **61**) with DAP Epimerase.



The enzyme E binds **56** to produce the Michaelis complex (E.**56**) which undergoes an irreversible loss of hydrogen fluoride to give the enzyme-enamine complex (E.**115**).

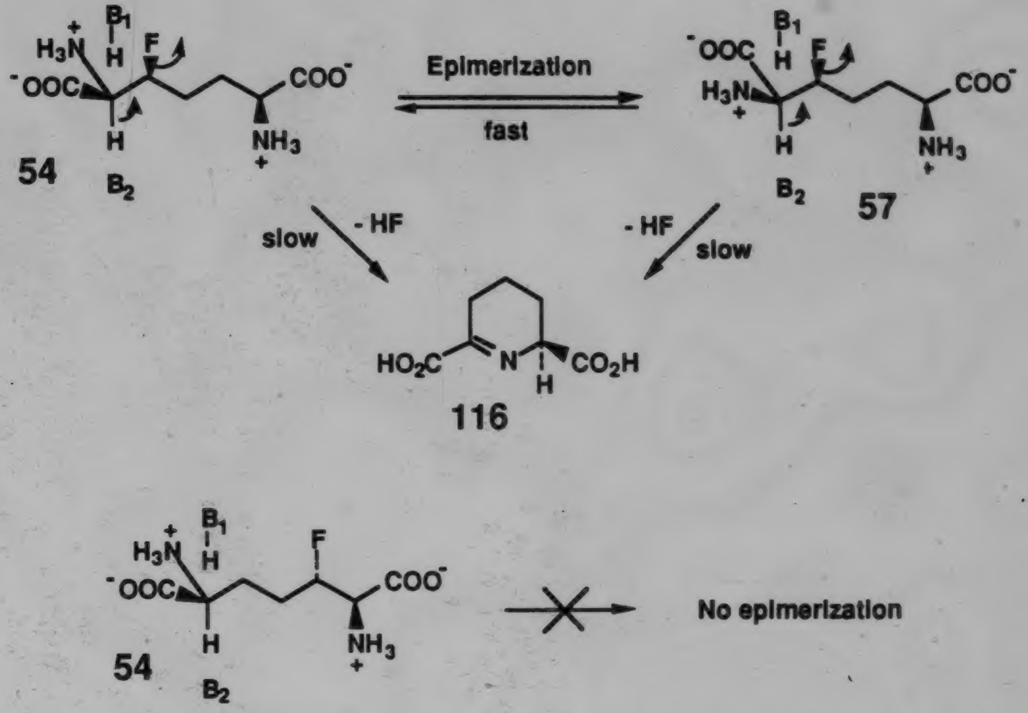
Product dissociation regenerates the free enzyme and eventually **115** cyclizes to form **116**. Since the isotope effect on V_{max} (1.1) is close to unity, the HF elimination step is believed to be very fast compared to the release of enamine **115** from the enzyme (i.e. $k_3 << k_2$) because only the elimination step is subject to isotope effect. Thus the potent inhibition of DAP-epimerase by **56** seems to be explained by a slow step that occurs after the loss of fluoride. This step is likely to be the release of enamine **115** from the enzyme.

The results in Table 3 indicate that the fluorinated DAP analogs **54**, **55**, **56**, and **57** are potent inhibitors of DAP epimerase. The initial rationale for this was that enzyme-catalyzed proton abstraction from the α-carbon of the DAP analog would be accompanied by elimination of an electronegative substituent from the adjacent β-carbon to generate the enzyme-bound enamine **115**. For enamine **115**, the α- and β-carbons as well as the attached α-amino and α-carboxyl groups are confined to a plane (Scheme 47). This enzyme-bound intermediate might therefore be a structural mimic of the planar transition state that is formed in the enzyme-catalyzed epimerization of the DAP substrate (Figure III). Based on the concept of transition state stabilization by the enzyme,[215] it might be expected that **115** would be tightly bound to the active site of DAP-epimerase and released very slowly into solution. This view is analogous to the inhibition of proline racemase by analogs that have a trigonal arrangement of atoms at the site of racemization.[216,217]

In addition to being slow substrate inhibitors of DAP epimerase, the fluorinated DAP analogs might also undergo enzyme-catalyzed epimerization. Epimerization reactions were examined and the experiments suggested that DAP-epimerase catalyzed the HF elimination from **56** without a competing epimerization reaction.

For isomer **55**, in principle, epimerization at either end of the molecule is possible. Upon the action of DAP epimerase, **55** underwent hydrogen fluoride elimination reaction as fast as **56** did. Along with this, a small amount of epimerization at the non-fluorinated end of **55** was also detected. This result is consistent with the epimerization of hydroxy DAP analog **29** which also occurred on the unsubstituted end (Scheme 48).

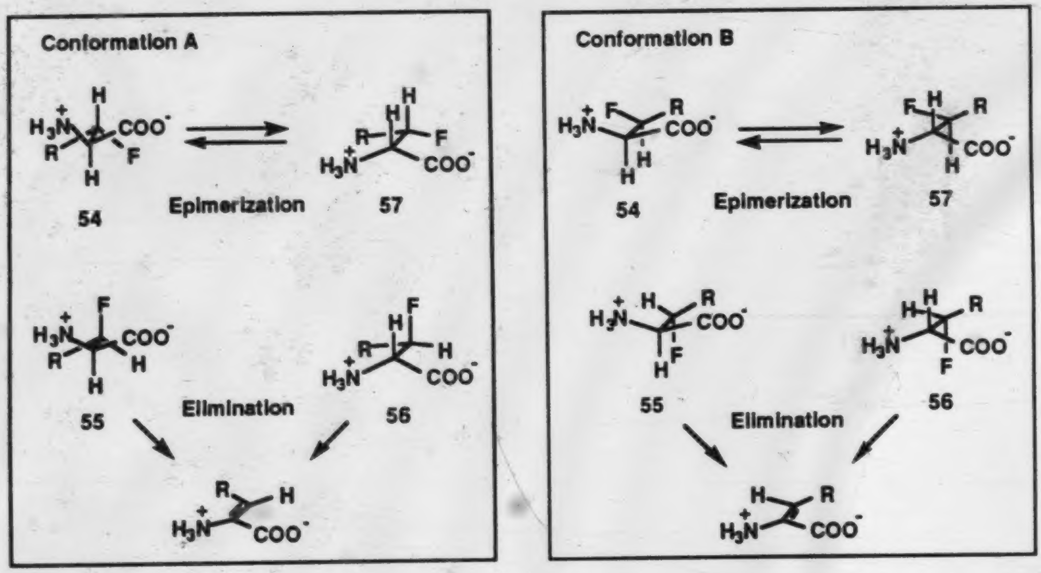
Scheme 50



In principle, the epimerization of **54** can occur at either end of the molecule. Epimerization at the fluorinated end of **54** was observed on the ^{19}F NMR spectrum to generate isomer **57** (Scheme 50). Surprisingly, epimerization at the non-fluorinated end of **54** did not occur. This result is probably due to a steric effect of the fluorine which prevents the binding of **54** with the non-fluorinated end in a catalytically productive site of the epimerase. This idea is not inconsistent with the results described above in which the epimerizations of **55** and of the hydroxy analog **29** occur on the unsubstituted end. For isomer **54**, the fluorine lies on the opposite side of the DAP skeleton compared to the substituent in analogs **55** and **29**. This degree of substrate specificity expressed by DAP epimerase is remarkable in that the replacement of a single hydrogen by fluorine at C-3 is sufficient to prevent the epimerization three carbons away at C-6.

The results in Table 3 indicate that the maximal velocities for the enzymatic elimination of HF from the fluoro DAP analogs fall into two groups. Compounds **55** and **56** undergo elimination much more rapidly than compounds **54** and **57**. An attempt to explain these results is illustrated in Figure 28. Since the elimination reaction is fast for isomer **55**, it is reasonable to propose that its conformation when bound to the epimerase is such that the α-hydrogen and β-fluorine atoms are oriented in either an anti-coplanar (Figure 28, conformation A) or in a syn-coplanar (Figure 28, conformation B) fashion. According to stereoelectronic theory,[63] these two geometries are predicted to lead to facile elimination of HF. Wiseman and Nichols have suggested that the epimerization of DAP occurs by a two base mechanism in which one enzymic base removes a proton from the α-carbon and an enzymic acid delivers a proton to the opposite face of the substrate (Figure

Figure 28 Possible Conformations of Fluoro DAP Analogues Bound to DAP Epimerase



III).[47] In addition, there must be a binding pocket on the enzyme for the distal amino and carboxyl groups of the substrate. This is based on the rigid stereochemical requirements of the epimerase which only accepts isomers of DAP with the L-configuration at the distal end. If **55** is bound with the eliminating groups in an anti-coplanar fashion (conformation A), then the remaining portion of the substrate (designated R in Figure 28) would be positioned on the same side as the 2-amino group. Alternatively, a syn-coplanar arrangement of the eliminating groups of **55** (conformation B) would place the R-group on the opposite side of the 2-amino group (Figure 28). Since the structure of the bound inhibitor is not known, these possibilities cannot be distinguished at the present time. However, it is likely that all of the fluorinated DAP isomers bind in a manner that maintains the same relative position of the charged functional groups on the enzyme surface. Based on these arguments, it is possible to predict the relative ease of HF elimination for the other three fluorinated DAP isomers examined in this study. Facile elimination is predicted for isomer **56** and this was found to be the case. In contrast, binding of both **54** and **57** to the enzyme would leave the eliminating groups in a gauche conformation (Figure 28) which is not favorable for elimination. Again, this is consistent with the results in Table 3.

Interestingly, when the elimination of HF is a facile process (isomers **55** and **56**) no competing epimerization at the carbon adjacent to fluorine is seen. In contrast, when hydrogen fluoride loss is disfavored (isomers **54** and **57**) epimerization at the α-carbon adjacent to the fluorinated carbon occurs readily.

(2) Preliminary Tests for Antibacterial Activity of Fluorinated Diaminopimelates

(The antibacterial tests described in this section and in the following Section 6 were accomplished by Professor Michael A. Pickard in Department of Microbiology, University of Alberta)

Compounds **54** and **56** were individually tested for antimicrobial activity in defined liquid media (with ammonium nitrate as nitrogen source) using wild-type organisms: *Bacillus subtilis* 6051; *Bacillus megaterium* 15374; *Escherichia coli* 9637; *Pseudomonas aeruginosa* 10145; *Salmonella typhimurium*; and *Serratia marcescens*. Optical density readings of overnight growth showed that within experimental error (10 %) no antibiotic activity could be detected except against *B. megaterium* which is inhibited by **56** the extent of about 14 % at a concentration of 5mg/mL, 20 % at 20 mg/mL, and 50 % at 100 mg/mL. The inhibition is relieved by addition of DAP as a mixture of isomers (100 mg/mL). Compound **54** showed no inhibition. These results were initially surprising in view of the previously reported antibiotic activity of mixtures of isomers of β-fluorodiaminopimelates and β-chlorodiaminopimelates.[20,21] However, some of the earlier studies have employed *E. coli* mutants which were DAP auxotrophs. In addition, it is likely that transport of DAP analogs into the bacterial cell is variable, and may be quite poor in many cases.[16] This problem could potentially be overcome by formation of di- or tripeptides which could exploit the peptide transport systems and be subsequently cleaved within the bacterial cell by peptidases.[16]

(3) Inhibition of DAP decarboxylase by DAP phosphonates

(This enzyme inhibition study was done by Professor Monica M. Palcic and Suzanne Crawley in the Department of Food Science, University of Alberta)

DAP phosphonate analogues **92**, **93**, **94**, **95**, **98** were tested as inhibitors for DAP decarboxylase, and the results (shown in Table 4) indicate that compound **93** was a moderate inhibitor of the decarboxylase while the remaining compounds exhibited very weak inhibition.

Table 4 Inhibition of DAP Decarboxylase by DAP Phosphonate Analogs.

Concentration / Compound	% Activity remaining	
	320 μM	560 μM
92	89	89
93	50	45
94	93	91
95	91	81
98	84	87

(4) Inhibition of DAP epimerase by DAP phosphonates

(The enzyme inhibition studies described in this section and in the following section were performed by Patricia M. Lane-Bell and Dr. Lister K.P. Lam in our research group)

Compounds **92**, **93**, **94**, **95**, **98** were examined for inhibition of DAP epimerase, and the results (shown in Table 5) showed that **94** was not an inhibitor of the epimerase whereas analogues **92**, **93**, **95**, **98** were found to be weak competitive inhibitors of the enzyme.

Table 5 Inhibition of DAP Epimerase by DAP Phosphonate Analogs

Compound	Concentration (mM)	Ki (mM)
92	3.0	6.2
93	0.3, 3.0	NI, 3.9
94	3.0	NI
95	3.0	7.2
98	3.0	4.2

(5) Inhibition of meso-DAP dehydrogenase by DAP phosphonates

The DAP phosphonate analogues **92**, **93**, **94**, **95** and **98** were found not to be substrates for the dehydrogenase, and **93**, **94**, **95** and **98** were shown to be weak competitive inhibitors of the dehydrogenase (for **93**, **95** and **94**, Ki = 4.3, 7.4 and 26 mM respectively). Analogue **92** was a weak noncompetitive inhibitor (Ki' = 27 mM) of the enzyme.

(6) Antibacterial activity of DAP phosphonates and their dipeptides

(These studies were done by Professor Pickard)

Compounds **93**,**92**, **95**,**94**,**103**,**100**, **102** and **101** were tested as individual isomers for antimicrobial activity in defined liquid media (using ammonium sulfate as a nitrogen source) with wild-type bacteria: *Bacillus subtilis* ATCC 6051, *B. megaterium* ATCC 15374, *E. coli* ATCC 9637, *Pseudomonas aeruginosa* ATCC10145, *Salmonella typhimurium*. Optical density readings of growth at 6 hours and 24 hours showed that, within experimental error (10%), only negligible or low antibiotic activities were detected with the following exceptions. With peptide **100** , *B. megaterium* was inhibited 53% at 100 ug/ ml in 6 hours.This inhibition was no longer present at 24 hours. Peptide **102** inhibited *B. subtilis* 21% at 10ug/ml and 41% at 100ug/ml in 24 hours.

Experimental

General

All reactions requiring anhydrous conditions were performed under a positive pressure of dry Ar using oven-dried glassware (>12 h, 120 °C) which had been cooled under Ar. All organic layers obtained from extractions were dried over Na_2SO_4. Concentration of samples *in vacuo* refers to the removal of solvent on a rotary evaporator followed by evacuation (< 0.05mm Hg) to constant sample weight. All solid products were dried *in vacuo* over P_2O_5 or anhydrous $CaSO_4$, and usually at 56 °C (b.p. of acetone). Solvents for anhydrous reactions were dried according to Perrin *et al.*[218] Benzene, tetrahydrofuran (THF), diethyl ether and toluene were distilled from sodium and benzophenone. Acetonitrile, triethylamine, diisopropylamine and pyridine were distilled form calcium hydride. Anhydrous ethyl alcohol and methyl alcohol were prepared by distilling from magnesium metal with catalytic iodine. Solvents used for chromatography were distilled. Water used was Milli-Q (Millipore) quality, which when necessary was degassed under vacuum. Aqueous hydrochloric acid was prepared free of metal ions from Milli-Q quality water and glass distilled as constant boiling (*c.a.* 110 °C) 5.7 N HCl.

All reagents employed were ACS grade or finer. Air sensitive reagents were handled under an atmosphere of dry Ar. Commercial (diethylamino)sulfur trifluoride (DAST) (Aldrich) was distilled at reduced pressure before use (safety shield, 43-44 °C/8 mmHg). Commercial organometallic regents were obtained from Aldrich Chemical Co. n-Butyllithium solution was periodically titrated against menthol / phenanthroline.[219]Amino acids and amino acid derivatives used as starting materials were obtained from Sigma Chemical Co.

When possible, the progress of reactions was monitored by thin-layer chromatography (TLC) using one or more of the following methods for visualization: UV absorption by fluorescence quenching; iodine staining; bromocresol green spray for acids; ninhydrin spray for amino acids; dodecamolybdophosphoric acid spray. All spray reagents were prepared and used as described by Krebs *et al.*[221] For TLC of amino acids on ion exchange resin sodium citrate buffer (pH 5.1) was used as a solvent. For monitoring reactions in water or other non-volatile solvents, the solvent was removed from the plate *in vacuo* or by heating before developing.

Commercial thin layer and preparative layer chromatography plates were: normal silica, Merck 60 F-254; reverse-phase, Merck RP-8F_{254} S and Macherey-Nagel and Co.; ion exchange resin, Polygram^{R} Ionex-25 SA-Na. Silica gel for column chromatography was Merck type 60, 70-230 mesh or its equivalent from General Intermediates of Canada. Flash chromatography was performed according to Still *et al.*[222] using Merck type 60, 230-420 mesh silica gel. Normal phase medium pressure liquid chromatography (MPLC) used a column of Merck Kieselgel 60 H (*ca.* 55 g, 2.5 x 30 cm). Reverse phase MPLC was performed on Merck Lobar Lichroprep RP-8 columns. All solvent mixtures were listed as volume ratios, and all medium pressure liquid chromatography was performed using solvents which were previously degassed under vacuum. The cation exchange resin used was BioRad AG 50, 50 x 8 (H^+ form, 50-100 mesh).

High pressure liquid chromatography (HPLC) employed a Hewlett Packard 1082B instrument fitted with an amino column (8MBNH10μ) from Pharmacia Waters Co. and UV detector set at 200 nm. For preparative purposes on this instrument, a sample volume of 60 μL at a concentration of 40 mg/mL was injected each time. Acetonitrile and methanol used were of HPLC grade. Solvents used were previously degassed and filtered through filters (0.45 μm).

Cellulose powder used for cellulose chromatography was Merck type and was usually packed and run under a small pressure (3-5 psi). Compound detection in the fractions was by TLC and performed after removal of the solvents in each fraction on a speed \ concentrator. Lyophilization of water soluble samples was accomplished on a freeze dryer of Fluxi-dry™ µp.

All literature compounds had IR, ^{1}H NMR, and mass spectra consistent with the assigned structures. Melting points are uncorrected and determined on a Thomas Hoover or Buchi oil immersion apparatus using open capillary tubes. Optical rotations were measured on a Perkin Elmer 241 polarimeter with a microcell (10.00 cm, 0.9 mL) at ambient temperature. All specific rotations reported were measured at the sodium D line. Infrared spectra (IR) were recorded on a Nicolet 7199 FT-IR spectrometer. Mass spectra (MS) were recorded on a Kratos AEI MS-50 (high resolution, electron impact ionization), MS-12 (chemical (NH_3) ionization, CI-MS), MS-9 (fast atom bombardment with argon, POSFAB). The term 'Cleland's reagent' (as a matrix in FAB MS) refers to a 5:1 mixture of dithiothreitol and dithioerythritol. Microanalyses were obtained using a Perkin Elmer 240 CHN analyzer.

Nuclear magnetic resonance (NMR) spectra were measured on Bruker WP-80 (continuous wave), WH-200, AM-300, WM-360 or WH-400 instruments in the specified solvent with tetramethylsilane (TMS) or (DSS) in D_2O as internal standards for ^{1}H NMR spectra. For ^{13}C NMR spectra, which were obtained on the WH-200, AM-300 or WH-400, the deuterated solvent peak was used as the reference. ^{19}F NMR spectra were recorded at 376 MHz using $CDCl_3$ or D_2O solvent with $CFCl_3$ as an internal standard. ^{31}P NMR spectra were recorded at 80 MHz using 85% H_3PO_4 as external standard.

Diethyl phthalimidomalonate (1)

A modification of the literature procedure was used.[223] A vigorously stirred solution of diethylamino malonate hydrochloride (10.2 g, 48.0 mmol) in 0.5 M aqueous Na_2CO_3 (96 mL) was treated with N-ethoxycarbonyl phthalimide (10.5 g, 48.0 mmol). The mixture was then stirred at 20 °C for 12 h. The solution was acidified with 12 N HCl to pH 2-3. CH_2Cl_2 (200 mL) was added, and the two phases were separated. The aqueous layer was extracted with CH_2Cl_2 (2 x 100 mL). The combined CH_2Cl_2 solutions were washed with 1 N HCl (2 x 100 mL), dried over Na_2SO_4, and concentrated *in vacuo* to give an oil, which was purified by flash column chromatography using EtOAc / petroleum ether (50 : 50) to afford the pure product (8.46 g, 58 %). IR ($CHCl_3$ cast) 2980 (m), 1754 (s), 1727 (s), 1392 (s), 1250 (m), 1180 (m), 1110 (m), 1035 (m), 730 (m) cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 7.95-7.60 (m, 4 H, Ar*H*), 5.45 (s, 1 H, NC*H*CO_2), 4.28 (q, 4 H, J=7 Hz, 2 C$H_2$$CH_3$), 1.28 (t, 6 H, J=7 Hz, 2 CH_2CH_3); MS 305.0896 (305.0899 calcd for $C_{15}H_{15}NO_6$); Anal. calcd for $C_{15}H_{15}NO_6$: C, 59.02; H, 4.92; N, 4.59. Found: C, 59.08; H, 4.83; N, 4.54.

4-Phthalimido-4,4-dicarbethoxybutyraldehyde (2)

The literature procedure was modified.[224] A solution of absolute ethanol (10 mL) and sodium metal (0.13 mmol) was mixed with diethyl phthalimidomalonate (**1**) (0.805g, 2.64 mmol) and the resulting mixture was cooled to 0 °C. Acrolein (0.20 mL) was then added dropwise. After the addition, the reaction mixture was brought to 20 °C for 15 min. It was then neutralized by glacial acetic acid (0.05 mL), and the solvents were removed *in vacuo*. The residue was partitioned between water and CH_2Cl_2. The organic layer was separated, dried over Na_2SO_4, and concentrated *in vacuo* to give an oil, which was purified by flash chromatography using EtOAc / petroleum ether (50 : 50) to give the pure product (0.410 g, 43 %). IR ($CHCl_3$ cast) 2980 (m), 1784 (s), 1752 (s), 1725 (s), 1371 (s), 1259 (s), 1192 (s), 1145 (s), 1030 (m), 875 (m), 720 (s) cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 9.71 (s, 1 H, C*H*O), 8.00-7.60 (m, 4 H, Ar*H*), 4.30 (q, 4 H, J=7 Hz, 2 C$H_2$$CH_3$), 3.05-2.55 (m, 4 H, 2 CH_2CH_2), 1.30 (t, 6 H, J=7 Hz, 2 CH_2CH_3); MS 361.1176 (361.1162 calcd for $C_{18}H_{19}NO_7$); Anal. calcd for $C_{18}H_{19}NO_7$: C, 59.83; H, 5.26; N, 3.88. Found: C, 59.57; H, 5.20; N, 3.85.

Copper Glycinate (3)

The literature procedure was modified.[116] An aqueous solution of glycine (10.5 g, 0.142 mol) (200 mL) was heated to reflux with cupric carbonate (12.0 g, 97 mmol) for 3 h. The excess of the latter was filtered from the hot solution. The filtrate was concentrated *in vacuo*, cooled, and the resulting copper glycinate crystals (4.02 g, 25% yield) were collected by vacuum filtration.

2-Amino-3-hydroxy-6-phthalimido-6,6-dicarbethoxyhexanoic Acid (4) and 3-Hydroxy-2,6-diaminopimelic Acid (5)

The literature procedure was modified.[108] To a stirred mixture of compound **2** (4.00 g, 11.1 mmol), copper glycinate (**3**) (1.22 g, 5.36 mmol), 4 N NaOH (8.5 mL, 34.0 mmol) and ice-water (30 mL), was added with ethanol until the aldehyde **2** just dissolved. The mixture was then stirred at 7-10 °C for 48 h. It was acidified with 12 N HCl to pH 3-4 and the copper was precipitated as copper sulfide by H_2S gas. The precipitate was filtered and the filtrate was concentrated *in vacuo*. The residue was applied to an ion exchange column (AG 50W-X8, H^+ form, 2.6 x 14 cm, 80 mL bed volume), which was eluted with 1N HCl. The ninhydrin positive fractions were concentrated *in vacuo* to give a white amorphous solid compound **4**, which was dissolved in 6 N HCl (60 mL), and heated to reflux for 10 h. The solvent was removed *in vacuo*, the residue was then applied to an ion exchange column (AG 50W-X8, H^+ form). The desired amino acid was eluted with 1 N HCl (0.56 g, yield: 19 %). A part of this was further purified by HPLC (NH_2 column) using 0.01M KH_2PO_4 aqueous buffer (pH 4.3) and CH_3CN / H_2O (500: 70, v / v). IR (neat) 3600-2400 (s), 1744 (s), 1590 (m), 1490 (s), 1420 (s), 1215 (s) cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 4.18-4.04 (m, 1 H, C*H*OH), 3.82 (t, 1 H, J=6 Hz, CH_2CHNH_2), 3.86 and 3.66 (m, 1 H, $OCHCHNH_2$), 2.26-1.56 (m, 4 H, CH_2CH_2); FAB-MS 207 (MH^+) (206.0903 calcd for $C_7H_{14}N_2O_5$).

Dimethyl L-Glutamate· HCl (6)

To a suspension of L-glutamic acid (20.0 g, 0.136 mol) in dry MeOH (300 mL) at 0 °C was added anhydrous HCl gas until saturation was reached. The reaction mixture was stirred at 20 °C for 1 day. The solvent was removed *in vacuo*, the residue was recrystallized from isopropanol / diisopropyl ether to give the pure product (26.6 g, 93%). IR ($CHCl_3$ cast) 3200-2500 (s), 1740 (s), 1505 (m), 1440 (s), 1233 (s) cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 8.63 (br s, 3 H, N*H*$_3$C), 4.38 (br s, 1 H, NC*H*CO$_2$), 3.83 (s, 3 H, OC*H*$_3$), 3.68 (s, 3 H, OC*H*$_3$), 3.00-2.15 (m, 4 H, C*H*$_2$C*H*$_2$); MS 143.0581 (M$^+$ - CH_3OH) (143.0582 calcd for $C_6H_9NO_3$). Anal. calcd. for $C_7H_{13}NO_4$: C, 47.99; H, 7.48; N 8.00. Found: C, 47.71; H, 7.33; N, 7.85.

Dimethyl (2S)-2-(4,5-diphenyl-2-oxo-4-oxazolin-3-yl)pentanedioate (7)

To a stirred solution of **6** (0.890 g, 4.20 mmol) and 4,5-diphenyl-1,3- dioxol-2-one (1.00 g, 4.20 mmol) in DMF (40 mL) at 20 °C, was added dropwise Et_3N (1.00 g, 10.0 mmol). The reaction mixture was stirred at 20 °C for 3 days. EtOAc (50 mL) was added and the solution was washed three times with water (3 x 50 mL). The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was taken up in trifluoroacetic acid (15 mL) and stirred at 20 °C for 6 h. The trifluoroacetic acid was removed *in vacuo* and the residue was dissolved in CH_2Cl_2 (60 mL). The CH_2Cl_2 layer was washed with water (50 mL), dried over Na_2SO_4, and concentrated *in vacuo* to give an oil. This was purified by flash chromatography using EtOAc / Hexane (40 / 60). The pure product was obtained in 11% yield (0.182 g). IR ($CHCl_3$ cast) 1763 (s), 1743 (s), 1360 (s), 1210 (m) cm^{-1}; ^{1}H NMR (80 MHz, $CDCL_3$) δ 7.65-7.00 (m, 10 H, Ar*H*), 4.30 (t, 1 H, NC*H*COO), 3.75 (s, 3 H, C*H*$_3$O), 3.55 (s, 3 H, OC*H*$_3$), 2.65-2.15 (m, 4 H, C*H*$_2$C*H*$_2$); MS 395.1365 (395.1369 calcd for $C_{22}H_{21}NO_6$). Anal. calcd for $C_{22}H_{21}NO_6$: C, 66.83; H, 5.35; N, 3.54. Found: C, 66.71; H, 5.31; N, 3.49.

(2S)-2-(4,5-Diphenyl-2-oxo-4-oxazolin-3-yl) 1,5-pentanediol (8)

Lithium borohydride (10.0 mg, 0.459 mmol) was added to a solution of **7** (0.160 g, 0.405 mmol) in dry THF (10 mL). The mixture was protected from light and stirred at 20 °C for 13 h. It was then heated to reflux for 4 h. Water was used to quench the reaction. The solvent was removed *in vacuo* and the aqueous layer was extracted three times with CH_2Cl_2 (3 x 40 mL). The combined CH_2Cl_2 solutions were dried over Na_2SO_4 and concentrated *in vacuo* to give an oil. This was purified by preparative TLC to give **8** (52.0 mg) in 38% yield. For **8**: IR (CH_2Cl_2 cast) 3600-3100 (s), 2941 (s), 2878 (m), 1728 (s), 1450 (m), 1274 (m), 1054 (m), 701 (m) cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 7.70-7.00 (m, 10H, Ar*H*), 4.25-3.38 (m, 5H, 2 $HOCH_2$ and NC*H*), 2.56 (br s, 2H, 2 *H*OCH_2), 2.15-1.25 (m, 4H, CH_2CH_2); MS 339.1469 (339.1470 calcd. for $C_{20}H_{21}NO_4$).

L-Glutamic Acid γ-Methyl Ester (9)

The literature procedure was modified.[132] L-glutamic acid (36.8 g, 0.250 mol) was added to a mixture of acetyl chloride (22.1 g, 0.281 mol) in methanol (250 mL) at 0 °C with vigorous stirring. After 5 min, the mixture was warmed to 20 °C and stirred for 20 h. Pyridine (25 mL) was added causing white crystals to precipitate. The mixture was stirred at 20 °C for 2 additional days. The solid was collected by vacuum filtration and washed with ethanol and ether (16.6 g, 40%). mp 185-187 °C; (lit.mp[132] 182 °C) IR (KBr) 3500-2400 (s), 1731 (s), 1612 (s), 1579 (s), 1413 (s), 1355 (m), 1328 (s), 1201 (s), 1182 (s) cm^{-1}; ^1H NMR (360 MHz, D_2O) δ 3.78 (t, 1 H, J=6.5 Hz, NC*H*COO), 3.72 (s, 3 H, CH_3O), 2.57 (td, 2 H, J =7.9 Hz, 4.3 Hz, $CH_3O_2CCH_2$), 2.16 (m, 2 H, CH_2CH_2CH); ^{13}C NMR (75.47 MHz, D_2O) (^1H decoupled) δ 175.38 (*C*OO), 173.94 (*C*OO), 54.03 (N*C*HCO), 52.41 (OCH_3), 29.75 (MeO_2CCH_2), 25.50 ($CH_2CH_2CH_2$); MS 143 (M^+ - H_2O) (143.0582 calcd for $C_6H_9NO_3$). Anal. calcd for $C_6H_{11}NO_4$: C, 44.72; H, 6.88; N, 8.69. Found: C, 44.91; H, 6.81; N, 8.64.

N-(tert-Butoxycarbonyl)-L-glutamic Acid γ-Methyl Ester (10)

To a solution of compound **9** (12.9 g, 80.0 mmol) in dioxane (160 mL), water (80 mL) and 1 N aqueous NaOH (80 mL) at 0 °C was added di-*tert*-butyl pyrocarbonate (19.2 g, 88.0 mmol). The mixture was stirred at 20 °C for 2 days. The solution was concentrated to 80 mL *in vacuo* and then neutralized with 1.3 N aqueous $NaHSO_4$ to pH 2-3 with cooling to 0 °C. The product was extracted with EtOAc (3 x 100 mL) three times. The combined EtOAc solutions were dried over Na_2SO_4 and concentrated *in vacuo* to give an oil (3.76 g, 18%). IR ($CHCl_3$ cast) 3400-2400 (s), 1736 (s), 1718 (s), 1520 (m), 1369 (m), 1253 (s), 1204 (s), 1166 (s), 1055 (m) cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 10.30 (s, 1 H, COO*H*), 5.30 (br s, 1 H, *H*N), 4.30 (br s, 1 H, NC*H*CO), 3.68 (s, 3 H, C*H*$_3$O), 2.60-1.85 (m, 4 H, C*H*$_2$C*H*$_2$), 1.45 (s, 9 H, (C*H*$_3$)$_3$C); MS 205. 0586 (M^+ - C_4H_8) (205.0586 calcd for $C_7H_{11}NO_6$). Anal. calcd. for $C_{11}H_{19}NO_6$: C, 50.57; H, 7.33; N, 5.36; Found: C, 50.20; H, 7.21; N, 5.27.

N-(tert-Butoxycarbonyl)-L-glutamic Acid α-tert-Butyl-γ-methyl Diester (11)

To a mixture of compound **10** (0.261 g, 1.00 mmol) and concentrated H_2SO_4 (10 μL) in dry CH_2Cl_2 (10 mL), was introduced isobutylene gas until saturation was reached. The reaction mixture was maintained at 20 °C for 6 days. It was then washed with 1 N aqueous Na_2CO_3 (20 mL) and water (20 mL). The organic phase was dried over Na_2SO_4 and concentrated *in vacuo* to afford an oil (40.1 mg, yield: 12%). IR ($CHCl_3$ cast) 3380 (m), 2970 (s), 1738 (s), 1718 (s), 1510 (m), 1368 (s), 1157 (s) cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 5.10 (br s, 1 H, *H*N), 4.25 (br s, 1 H, NC*H*CO), 3.70 (s, 3 H, C*H*$_3$O), 2.60-1.50 (m, 4 H, C*H*$_2$C*H*$_2$), 1.45 (s, 18H, 2 (C*H*$_3$)$_3$C); MS 216.1236 (M^+ - C_4H_9 - CO_2) (216.1236 calcd for $C_{10}H_{18}NO_4$).

N-(Benzyloxycarbonyl)-L-glutamic acid (12)

The literature procedure was modified.[225] To a stirred mixture of $NaHCO_3$ (58.8 g, 0.700 mol) and L-glutamic acid (29.4 g, 0.22 mol) in water (350 mL) at 20 °C, was added benzyloxycarbonyl chloride (37.4 g, 0.220 mol) over a period of 30 min. The solution was then stirred at 20 °C for 24 h. The aqueous solution was washed with ether (2 x 60 mL) and acidified to congo red with 12N HCl. The product was extracted with EtOAc (3 x 150 mL). The combined EtOAc phases were dried over Na_2SO_4 and concentrated *in vacuo* to afford a sticky oil (43.5 g, 78%). IR ($CHCl_3$ cast) 3400-2600 (br, s), 1711 (s), 1530 (m), 1410 (m), 1238 (s), 1060 (m), 750 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.18 (br s, 2 H, 2 COO*H*), 7.29 (s, 5 H, Ar*H*), 5.58 (d, 1 H, J=8 Hz, *H*NCO), 5.06 (s, 2 H, C*H*$_2$Ph), 4.42 (dt, 1 H, J=6 Hz, 8 Hz; NC*H*COO), 2.43 (m, 2 H, OOCC*H*$_2$), 2.14 (m, 2 H, CH_2C*H*$_2$CH); MS 281.0904 (281.0899 calcd for $C_{13}H_{15}NO_6$); Anal. calcd for $C_{13}H_{15}NO_6$: C, 55.52; H, 5.34; N, 4.98. Found: C, 55.12; H, 5.27; N, 4.90.

(S)-3-(Benzyloxycarbonyl)-4-(2-carboxyethyl)-5-oxazolidinone (13)

The literature procedure was modified.[226] A mixture of N-Cbz-L-glutamic acid (**12**)(38.8 g, 0.138 mol), paraformaldehyde (7.31 g, 0.244 mol), and *p*-toluenesulfonic acid (1.46 g, 7.68 mmol) in benzene (1200 mL) was heated at reflux for 7 h with the liberated water being removed azeotropically by means of a Dean-Stark apparatus. The solution was washed with water (2 x 500 mL) and extracted with 5% aqueous $NaHCO_3$ (600 mL). The extract was acidified with 6N HCl to pH 1-2, and extracted with EtOAc (3 x 250 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to offer an oil (32.0 g, 79%). IR ($CHCl_3$ cast) 3500 (m), 1802 (s), 1714 (s), 1417 (s), 1360 (m), 1245 (m), 1165 (m), 1050 (m), 765 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.96 (br s, 1 H, *H*OOC), 7.38 (s, 5 H, Ar*H*), 5.54 (s, 1 H, NC*H*$_2$O), 5.22 (s, 1 H, NC*H*$_2$O), 5.20 (s, 2 H, C*H*$_2$Ph), 4.40 (t, 1 H, J=5.6 Hz,NC*H*CO), 2.60-2.06 (m, 4 H, C*H*$_2$C*H*$_2$); MS 293.0897 (293.0899 calcd for $C_{14}H_{15}NO_6$); Anal. calcd for $C_{14}H_{15}NO_6$: C, 57.34; H, 5.12; N, 4.78. Found: C, 57.07; H, 5.15; N, 4.73.

(S)-3-(Benzyloxycarbonyl)-4-(2-chlorocarbonylethyl)-5-oxazolidinone (14)

The literature procedure was modified.[227] To a solution of **13** (19.5 g, 66.6 mmol) in dry benzene (100 mL) at 0 °C, was added PCl_5 (13.9 g, 66.6 mmol). The mixture was stirred at 0 °C for 2 h during which time it became homogeneous. The solvent was removed *in vacuo*, and $POCl_3$ byproduct was azeotropically removed with dry toluene (2 x 25 mL) to give an amorphous solid (18.0 g, 87%). IR ($CHCl_3$ cast) 2980 (m), 1800 (s), 1710 (s), 1400 (s), 1350 (m), 1130 (m), 1060-970 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.38 (s, 5 H, Ar*H*), 5.52 (s, 1 H, NCH_2O), 5.22 (s, 1 H, NCH_2O), 5.20 (s, 2 H, CH_2Ph), 4.34 (t, 1 H, J=6.5 Hz, NC*H*CO), 3.05 (t, 2 H, J=6.8 Hz, $ClOCCH_2$), 2.24 (m, 2 H, CH_2CH_2CH); MS 275.0795 (M^+ - HCl) (275.0794 calcd for $C_{14}H_{13}NO_5$).

(S)-3-(Benzyloxycarbonyl)-4-(3-oxopropyl)-5-oxazolidinone (15)

The literature procedure was modified.[145] To a solution of **14** (10.0 g, 32.1 mmol) in dry THF (60 mL) at -78 °C was added LiAl(O-*t*-Bu)$_3$H (6.53 g, 25.7 mmol) in dry THF (80 mL) over a period of 2.5 h. The reaction mixture was then stirred at -78 °C for another 3 h. Water (20 mL) was added and the solvent was removed *in vacuo*. The residue was dissolved in dichloromethane and the mixture was filtered. The filtrate was extracted with water and 5% aqueous $NaHCO_3$ solution (100 mL). The organic phase was dried (Na_2SO_4) and concentrated *in vacuo* to give the aldehyde **15** (6.52 g, 73%) which could be used without further purification: IR ($CHCl_3$ cast) 2920 (m), 1800 (s), 1720 (s), 1410 (s), 1360 (m), 1040 (m), 760 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.68 (s, 1 H, C*H*O), 7.36 (s, 5 H, Ar*H*), 5.55 (m, 1 H, OCH*H*N), 5.22 (m, 3 H, OCH*H*N and CH_2Ph), 4.38 (t, 1 H, 6 Hz, NC*H*CO), 2.70-2.10 (m, 4 H, CH_2CH_2); MS 277.0947 (277.0950 calcd for $C_{14}H_{15}NO_5$). Anal. Calcd for $C_{14}H_{15}NO_5$: C, 60.65; H, 5.45; N, 5.05. Found: C, 60.71; H, 5.31; N, 5.03.

(S)-3-(Benzyloxycarbonyl)-4-(3-hydroxypropyl)-5-oxazolidinone (16)

To a solution of the acyl chloride **14** (1.56 g, 5.00 mmol) in dry THF (20 mL) at -78 °C was added dropwise lithium tert-butoxyaluminum hydride (2.69 g, 10.6 mmol) in dry THF (30 mL) over a period of 30 min. The mixture was stirred at -78 °C for another 4 h. The reaction was quenched with 1N HOAc (60 mL) at -78 °C. The organic solvent was removed *in vacuo* and the remaining aqueous solution was extracted with CH_2Cl_2 (3 x 100 mL). The combined CH_2CH_2 solutions were dried over Na_2SO_4 and concentrated *in vacuo* to give an oil, which was purified by flash chromatography using EtOAc / hexane (80 / 20) to yield pure **16**. (1.11 g, 78%). IR (CH_2Cl_2 cast) 3470 (s), 2933 (s), 1712 (s), 1418 (s), 1359 (s), 1247 (s), 1132 (s), 1052 (s), 767 (s), 700 (s) cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 7.40 (s, 5 H, Ar*H*), 5.40 (AB, 2 H, J=4 Hz, NC*H*$_2$O), 5.23 (s, 2 H, C*H*$_2$Ph), 4.40 (t, 1 H, J=5.3 Hz, NC*H*CO), 3.63 (br t, 2 H, HOC*H*$_2$), 2.40-1.40 (m, 4 H, C*H*$_2$C*H*$_2$); MS 279.1099 (279.1107 calcd for $C_{14}H_{17}NO_5$).

Bis(triphenylphosphine)copper Cyanoborohydride Dimer (17)

The literature procedure was modified.[57] Hydrated copper sulfate (4.00 g, 16.0 mmol) was stirred for 1h in a solution of triphenylphosphine (13.5 g, 50.0 mmol) in ethanol (350 mL) to give a pale green solution. Finely powdered sodium cyanoborohydride (5.03 g, 80.0 mmol) was added with stirring. Hydrogen was evolved and a white precipitate formed. After 1h, the crude product was collected by filtration and dissolved in $CHCl_3$ (100 mL). The insoluble material was removed by filtration and ethanol (200 mL) was added to the filtrate to induce crystallization. The white precipitate was collected by filtration, and washed with ethanol and ether to give compound **17** in 92% yield (9.22 g).

N-Methyl-2-Aminoethanamide (18)

The literature procedure was modified.[146] To a solution of CH_3NH_2 (41.0 g, 1.32 mol) in absolute ethanol (150 mL) cooled at 0 °C was added ethyl glycinate hydrochloride (10.0 g, 71.6 mmol) in ethanol (1500 mL). The reaction mixture was stirred at 0 °C for 4 h, then warmed to 20 °C, and stirred for 38 h. The mixture was concentrated to 150 mL *in vacuo* and ether (350 mL) was added to induce precipitation of $CH_3NH_2 \cdot HCl$ upon cooling the solution to -20 °C for 2 h. The $CH_3NH_2 \cdot HCl$ produced was removed by filtration, and the filtrate was concentrated *in vacuo* to give an oil in quantitative yield (6.74 g). IR ($CHCl_3$ cast) 3290 (s), 3080 (s), 2940 (s), 1662 (s), 1550 (s), 1411 (s), 1310 (m), 1275 (m), 1160 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.26 (br s, 1 H, *H*NCO), 3.35 (s, 2 H, NH_2CH_2), 2.85 (d, 3 H, J=4.5 Hz, NCH_3), 1.51 (s, 2 H, NH_2); MS 88.0646 (88.0637 calcd for $C_3H_8N_2O$). Anal. calcd. for $C_3H_8N_2O$: C, 40.90; H, 9.15; N, 31.79. Found: C, 40.73; H, 8.97; N, 31.80.

N-Methyl-2-[(2,2-dimethylpropylidene)amino]ethanamide (19)

The literature procedure was modified.[146] A solution of **18** (6.50 g, 73.8 mmol) in pentane (100 mL) was mixed with pivalaldehyde (6.35 g, 73.7 mmol). The reaction mixture was heated to reflux using a Dean-Stark apparatus to remove water. After a further 3.5 h, the solution was concentrated *in vacuo* to give a liquid. (8.81 g, 76.5 %). IR ($CHCl_3$ cast) 3322 (s), 2960 (s), 2837 (m), 1679 (s), 1535 (s), 1411 (s), 1364 (m), 1308 (m), 1264 (m), 1158 (m) cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 7.63 (s, 1 H, *H*C=N), 6.88 (br s, 1 H, *H*NCO), 4.05 (s,2 H, NCH_2CO), 2.90 (d, 3 H, J=4.6 Hz, $HNCH_3$), 1.10 (s, 9 H, $(CH_3)_3C$); MS 99.0559 (M^+ - C_4H_9) (99.0558 calcd for $C_4H_7N_2O$). Anal. calcd. for $C_8H_{16}N_2O$: C, 61.51; H, 10.32; N 17.93. Found: C, 61.42; H, 10.21; N, 17.84.

(R,S)-2-(1,1-Dimethylethyl)-3-methylimidazolidin-4-one (20)

The literature procedure was modified.[146] To a solution of compound **19** (8.73 g, 60.0 mmol) in methanol (30 mL) cooled at 0 °C was added methanol (50 mL) saturated with HCl gas. The reaction mixture was stirred at 0 °C for 30 min, then at 20 °C for 2 h. It was then concentrated *in vacuo* to give a white solid product (10.7 g) as a hydrochloride salt. To obtain the corresponding free amine, it was dissolved in CH_2Cl_2 (100 mL) and washed with 3 M NaOH (100 mL). This 3 M NaOH extract was then extracted with CH_2Cl_2 (50 mL). The combined organic phases were washed with water (2 x 50 mL) and dried over Na_2SO_4 to afford **20** (6.01 g, 69 %). IR (KBr) 3420 (m), 2990-2460 (s), 1711 (s), 1580 (m), 1480 (m), 1418 (s), 1400 (s), 1325 (m), 1030 (m), 750 (s), 680 (s) cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 4.15 (s, 1 H, NC*H*N), 3.48 (s, 2 H, NCH_2CO), 2.95 (s, 3 H, NCH_3), 2.03 (s, 1 H, N*H*), 0.95 (s, 9 H, $(CH_3)_3C$); MS 99.0559 (M^+ - C_4H_9) (99.0558 calcd for $C_4H_7N_2O$).

(2S, 2'S)-[2-(1,1-Dimethylethyl)-3-methyl-4-imidazolidin-1-yl] Mandelate (21), (2R, 2'S)-[2-(1,1-Dimethylethyl)-3-methyl-4-imidazolidin-1-yl] Mandelate (21a) and (2R)-1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinone (22)

The literature procedure was modified.[147] A mixture of compound **20** (6.00 g, 38.4 mmol) and (S)-(+) mandelic acid (6.00 g, 39.5 mmol) was dissolved in boiling acetone (15 mL). The solution was stirred to disperse the crystals in the flask, which was then cooled slowly to 20 °C over 6 h. This resulted in the separation of the (R) amine (S) mandelic acid salt. After 15 h in a refrigerator (5 °C), the crystals were filtered and dried *in vacuo* to give light yellow fine crystals **21** (4.00 g). The filtrate containing compound **21a** was set aside for other conversions.

A suspension of the (R, S) amine mandelic acid salt **21** (4.00 g, 13.0 mmol) in CH_2Cl_2 (30 mL) was shaken with 2 M NaOH (12 mL). After liquid-liquid separation, the aminal remaining in the CH_2Cl_2 layer was N-benzoylated by simultaneously additions of benzoyl chloride (1.79 g, 12.7 mmol) and 1 M NaOH (14.3 mL) with ice cooling. The reaction mixture was then stirred at 20 °C for 10 h. The two layers were separated and the CH_2Cl_2 phase was washed with water (50 mL), dried over Na_2SO_4, and concentrated *in vacuo* to afford a white crystalline **22** (3.00 g, 89 %). mp 143-144 °C (lit. mp[147] 143-144 °C); $[\alpha]_D$ - 116° (c 1, CH_2Cl_2) (lit.[147] $[\alpha]_D$= -126); IR (KBr) 2980 (s), 1713 (s), 1703 (s), 1647 (s), 1393 (s), 1381 (s), 1313 (m), 1237 (m), 794 (s), 722 (s), 698 (s) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.64-7.40 (m, 5 H, Ar*H*), 5.60 (s, 1 H, NC*H*N), 3.99 (AB, 2 H, J=16 Hz, NC*H*$_2$CO), 3.03 (s, 3 H, NC*H*$_3$), 1.06 (s, 9 H, (C*H*$_3$)$_3$C); FAB-MS 261 (MH^+) (260.1525 calcd for $C_{15}H_{20}N_2O_2$); Anal. calcd for $C_{15}H_{20}N_2O_2$: C, 69.23; H, 7.69; N, 10.77. Found: C, 68.95; H, 7.49; N, 10.49.

(2S)-1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinone (23)

The literature procedure was modified.[147] The filtrate containing compound **21a** was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (100 mL) and washed with 2 N NaOH (100 mL) and brine (100 mL).It was dried over Na_2SO_4, and concentrated *in vacuo* to give a yellowish liquid (2.19 g). To a solution of this (14.0 mmol) in CH_2Cl_2 (30 mL), were added benzoyl chloride (14.0 mmol) and 1N NaOH (16 mL) at 0 °C. The reaction mixture was stirred at 20 °C for 16 h. The two layers were separated and the CH_2Cl_2 layer was washed with brine (100 mL), dried over Na_2SO_4, and concentrated *in vacuo* to afford a yellow solid (3.44 g). This was recrystallized from ethanol (8-10 mL) to give white crystalline **23** (1.64 g). $[\alpha]_D$ +124 (c 1, CH_2Cl_2) (lit.[147] $[\alpha]_D$ +127); mp 143-144 °C (lit. mp[147] 143-144 °C); IR (KBr) 2983 (s), 1700 (s), 1645 (s), 1483 (m), 1449 (s), 1400 (s), 1364 (s), 1304 (s), 1253 (s), 796 (s), 721 (s) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.64-7.36 (m, 5 H, Ar*H*), 5.60 (s, 1 H, NC*H*N), 3.98 (AB, 2 H, J=16 Hz, NC*H*$_2$CO), 3.03 (s, 3 H, NC*H*$_3$), 1.06 (s, 9 H, (C*H*$_3$)$_3$C); CI-MS 261 (MH^+) (260.1525 calcd for $C_{15}H_{20}N_2O_2$); Anal. calcd for $C_{15}H_{20}N_2O_2$: C, 69.23; H, 7.69; N, 10.77. Found: C, 69.05; H, 7.77; N, 10.78.

(4S, 3'S, 2"R, 5"R)-4-[3-(1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinon-5-yl)-3-hydroxypropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (24) and (4S,3'S, 2"S, 5"R)-4-[3-(2-(1,1-Dimethylethyl)-3-

methyl-4-imidazolidinon-5-yl)-3-benzoyloxypropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (25)

A solution of lithium diisopropylamide (LDA) (1.56 mmol, 1.8 mL, prepared from diisopropylamine and *n*-BuLi) was slowly added to a stirred solution of (2R)-1-benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinone (**22**) (0.367 g, 1.41 mmol) in dry THF (10 mL) at -78 °C. The mixture was stirred at -78 °C for 40 min to afford a deep red solution. Aldehyde **15** (0.381 g, 1.37 mmol) in dry THF (8 mL) was added dropwise at -78 °C, and stirring was continued an additional 1.5 h. The mixture was treated with a half-saturated NH_4Cl solution (20 mL) and dichloromethane (20 mL) at -78 °C. The organic layer was separated, and the aqueous layer was extracted with dichloromethane (3 x 20 mL). The combined organic phases were dried (Na_2SO_4) and concentrated *in vacuo* to give an oil which was purified by flash chromatography (ethyl acetate:hexane, 60:40) to afford an amorphous solid (0.361 g, 49 % yield). This material consisted of an 88:12 mixture of **24** and a minor isomer **25** in which the N-benzoyl group had migrated to the hydroxyl oxygen: IR ($CHCl_3$ cast) 3420 (s), 2960 (m), 1800 (s), 1720 (s), 1690 (s), 1650 (m), 1410 (m), 1360 (m), 1260 (m), 700 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ (major isomer **24**, 88%) 7.66-7.30 (m, 10 H, 2 Ar*H*), 5.67 (s, 1 H, NC*H*NBz), 5.52 (br s, 1 H, CbzNCH*H*O), 5.26-5.14 (m, 3 H, NCH*H*O and CH_2Ph), 4.46 (d, 1 H, 4.0 Hz, OCC*H*NBz), 4.41 (d, 1 H, 12 Hz, O*H*), 4.24 (t, 1 H, 6 Hz, OCC*H*NCbz), 3.74 (m, 1 H, C*H*OH), 3.04 (s, 3 H, CH_3N), 2.12-1.90 (m, 2H, CH_2CH_2), 1.55-1.20 (m, 2 H, CH_2CH_2), 1.05 (s, 9 H, *t*-Bu); (minor isomer **25**, 12%) 8.18-8.00 (m, 8 H, Ar*H*), 7.96-7.88 (m, 2 H, Ar*H*), 5.57 (br s, 1 H, OCH*H*NCbz), 5.33-5.11 (m, 3 H, OC*H*HNCbz and CH_2Ph), 4.40 (t, 1 H, 6 Hz, OCC*H*NCbz), 4.19 (br s, 1 H, *t*-BuC*H*), 3.77 (br s, 1 H, OCC*H*NBz), 2.90 (s, CH_3N), 2.36-1.80 (m, 5 H, N*H* and CH_2CH_2), 0.98 (s, 9 H, *t*-Bu); MS 537.2440 (537.2474 calcd for $C_{29}H_{35}N_3O_7$). Anal. Calcd for $C_{29}H_{35}N_3O_7$: C, 64.80; H, 6.56; N, 7.82. Found: C, 65.10; H, 6.42; N, 7.48.

(2R, 3S, 6S)-3-Hydroxy-2,6-diaminopimelic Acid (26)

A mixture of **24** and **25** (0.709 g, 1.32 mmol) and 6 N HCl (50 mL) was heated at reflux for 12 h. The solution was cooled, extracted with dichloromethane (2 x 50 mL), and the aqueous phase was concentrated *in vacuo*. The residue was purified by ion exchange chromatography (AG 50-X8, H^+ form, 68 equiv bed volume) using water (100 mL) and then 1 N aqueous ammonia (300 mL). The ammonia fractions were concentrated *in vacuo* to afford pure **26** (0.272 g, 99%). The compound could be recrystallized from water/ethanol: mp 228-230 °C (dec); IR (KBr) 3640-2400 (br s), 1640 (s), 1610 (s), 1500 (s), 1410 (m), 1345 (m) cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 4.13-4.01 (m, 1 H, H-3), 3.79 (t, 1 H, 6 Hz, H-6), 3.65 (d, 1 H, 5.2 Hz, H-2), 2.20-1.54 (m, 4 H, CH_2CH_2); ^{13}C NMR (75.5 Hz, D_2O) δ 175.2, 173.5, 70.0, 60.1, 55.2, 30.1, 27.9; FAB MS (glycerol) 207 (MH^+).

(4S, 3'R, 2"S, 5"S)-4-[3-(1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinon-5-yl)-3-hydroxypropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (27) and (4S, 3'R, 2"R, 5"S)-4-[3-(2-(1,1-Dimethylethyl)-3-methyl-4-imidazolidinon-5-yl)-3-benzoyloxypropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (28)

The procedure described above to convert **22** to **24** and **25** was employed for condensation of (2S)-1-benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinone (**23**) (1.53 g, 5.88 mmol) with **15** (1.64 g, 5.92 mmol) to afford a 75:25 ratio of **27** and a minor isomer **28** in which the benzoyl group had migrated to the hydroxyl oxygen (1.60 g, 50 % yield): IR ($CHCl_3$ cast) 3400 (m), 2970 (m), 1800 (s), 1720 (s), 1690 (s), 1650 (s), 1410 (s), 1360 (s), 1260 (m), 760 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ (major isomer **27**, 75 %) 7.75-7.28 (m, 10 H, 2 Ar*H*), 5.65 (s, 1 H, NC*H*NBz), 5.51 (br s, 1 H, CbzNCH*H*O), 5.22-5.06 (m, 3 H, CbzNCH*H*O and CH_2Ph), 4.45 (d, 1 H, 4 Hz, OCC*H*NBz), 4.39 (d, 1 H, 12 Hz, O*H*), 4.22 (t, 1 H, 6 Hz, OCC*H*NCbz), 3.05 (s, 3 H,

$\mathrm{C}H_3\mathrm{N}$), 2.20-1.90 (m, 2 H, $\mathrm{CH_2C}H_2$), 1.50-1.20 (m, 2 H, $\mathrm{CH_2C}H_2$), 1.04 (s, 9 H, *t*-Bu); (minor isomer **28**, 25%) 8.06-7.99 (m, 2 H, Ar*H*), 7.68-7.58 (m, 8 H, Ar*H*), 5.51 (br s, 1 H, CbzNC*H*HO), 5.22-5.06 (m, 3 H, CbzNCH*H* and $\mathrm{C}H_2\mathrm{Ph}$), 4.22 (t, 1 H, 6 Hz, OCC*H*NCbz), 4.20 (s 1 H, *t*-BuC*H*), 2.89 (s, 3 H, $\mathrm{C}H_3\mathrm{N}$), 2.20-1.90 (m, 2 H, $\mathrm{C}H_2\mathrm{CH_2}$), 1.50-1.20 (m, 2 H, $\mathrm{CH_2C}H_2$), 0.98 (s, 9 H, *t*-Bu); MS 537.2465 (537.2474 calcd for $C_{29}H_{35}N_3O_7$); CI (NH_3) MS 538 (MH^+); Anal. calcd for $C_{29}H_{35}N_3O_7$: C, 64.80; H, 6.56; N, 7.82. Found: C, 64.65; H, 6.42; N, 7.62.

(2S, 3R, 6S)-3-Hydroxy-2,6-diaminopimelic Acid (29)

Compounds **27** and **28**, as a mixture, were hydrolyzed by the procedure described previously for the production of **26** from the mixture of **24** and **25**. A quantitative yield of **29** was obtained. IR (KBr) 3680-2400 (br s), 1630 (br s), 1507 (s), 1404 (m), 1350 (m) cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 4.16-4.04 (m, 1 H, H-3), 3.82 (t, 1 H, 6 Hz, H-6), 3.70 (d, 1 H, 5.2 Hz, H-2), 2.25-1.55 (m, 4 H, $\mathrm{C}H_2\mathrm{C}H_2$); ^{13}C NMR (75.5 Hz, D_2O) δ 175.2, 173.4, 70.1, 60.0, 55.3, 30.1, 27.9. FAB MS (glycerol) 207 (MH^+); Anal. calcd for $C_7H_{14}N_2O_5 \cdot 2\ HCl$: C, 30.11; H, 5.73; N, 10.03. Found: C, 29.90; H, 5.53; N, 9.95.

Treatment of the Mixture of 27 and 28 with DAST to Generate a Mixture of (4S,3'S, 2"R, 5"S)-4-[3-(2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinon-5-yl)-3-benzoyloxypropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (30) and Compound 28

The mixture of **27** and **28** was treated with DAST to give a mixture of compound **30** and **28**, by the procedure analogous to that used for the treatment of **24** and **25** with DAST to produce **32** and **25** as described below. IR ($CHCl_3$ cast) 3390 (m), 2960 (m), 1795 (s), 1720 (s), 1695 (s), 1652 (s), 1412 (s), 1365 (s), 1262 (m), 760 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) (minor isomer **30**, 40%) δ 8.06-7.78 (m, 2H, Ar*H*), 7.71-7.20

(m, 8H, Ar*H*), 5.53 (br d, 1H, J=4Hz, NCH_2O), 5.46-5.32 (m, 1H, *H*COCOPh), 5.28-5.06 (m, 3H, NCH_2O and OCH_2Ph), 4.37 (br t, 1H, NC*H*CO), 4.02 (d, 1H, J=2.5 Hz, NC*H*N), 3.90 (br s, 1H, NC*H*CO), 2.93 (s, 3H, CH_3N), 2.35-1.80 (m, 4H, CH_2CH_2), 1.60 (br s, 1H, N*H*), 0.91 (s, 9H, $(CH_3)_3C$); (major isomer **28**, 60%) δ 8.06-7.78 (m, 2H, Ar*H*), 7.71-7.20 (m, 8H, Ar*H*), 5.53 (br d, 1H, J= 4Hz, NCH_2O), 5.46-5.32 (m, 1H, *H*COCOPh), 5.28-5.06 (m, 3H, NCH_2O and OCH_2Ph), 4.37 (br t, 1H, NC*H*CO), 4.17 (d, 1H, J=2 Hz, NC*H*N), 3.81 (br s, 1H, NC*H*CO), 2.87 (s, 3H, CH_3N), 2.35-1.80 (m, 4H, CH_2CH_2), 1.60 (br s, 1H, N*H*), 0.99 (s, 9H, $(CH_3)_3C$); MS 537.2481 (537.2475 calcd for $C_{29}H_{35}N_3O_7$).

Hydrolysis of the Mixture of 30 and 28 to a Mixture of Compound (29) and (2S, 3S, 6S)-3-Hydroxy-2,6-diaminopimelic Acid (31)

The mixture of **30** and **28** (120 mg, 0.223 mmol) was dissolved in 6N HCl (30 mL) and the solution was heated at reflux for 4 h. After being cooled to 20 °C, the aqueous solution was washed with ether (2 x 40 mL) and concentrated *in vacuo* to give a solid residue, which was applied to an ion exchange column (AG 50W-8X, H^+ form, 10 mL bed volume). After being washed with water, the column was eluted with 1N NH_4OH. The eluent was concentrated *in vacuo* to afford a solid compound, which was further purified by a cellulose chromatography (2.5 x 29 cm) using nBuOH / H_2O /CH_3CO_2H (4 / 2 / 1). The pure product was obtained as dihydrochloride salt in 57% yield (35.7 mg). Its spectroscopic properties were identical to the mixture of **29** and **31** derived from the hydrolysis of **46** and **47** described below.

Hydrolysis of the Mixture of 46 and 47 to Compounds 29 and 31

Deprotection of the mixture of **46** and **47** (32.0 mg, 69.4 mmol) in 6N HCl (10 mL) by a procedure analogous to that described below for hydrolysis of **48** to **56** produced a mixture of **29** and **31** (18.2 mg, 94 % yield) as their hydrochloride salts. The sample showed identical chromatographic and spectral properties to **29** except for: ^{1}H NMR (300 MHz, D_2O, DSS) δ (major isomer, 55%, peaks enhanced if pure **29** added) 4.25 (m, 1 H, C*H*OH), 4.11 (t, 1 H, 6 Hz, CH_2C*H*N), 4.02 (d, 1 H, 5.2 Hz, C*H*NCHOH), 2.26-1.60 (m, 4H, CH_2CH_2); (minor isomer **31**, 45%) 4.25 (m, 1 H, C*H*OH), 4.14 (d, 1 H, 3.5 Hz, C*H*NCHOH), 4.11 (t, 1 H, 6 Hz, CH_2C*H*N), 2.26-1.60 (m, 4 H, CH_2CH_2); ^{13}C NMR (75.5 MHz, D_2O) δ (major isomer 55 %, peaks enhanced if pure **29** added) 172.75, 171.28, 69.23, 58.61, 53.44, 29.64, 27.30; (minor isomer **31**, 45 %) 172.75, 170.26, 69.88, 58.24, 53.60, 28.88, 27.58.

Treatment of the Mixture of 24 and 25 with DAST to Give a Mixture of (4S,3'R, 2"S, 5"R)-4-[3-(2-(1,1-Dimethylethyl)-3-methyl-4-imidazolidinon-5-yl)-3-benzoyloxypropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (32) and Compound 25

A solution of the mixture of **24** and **25** (56.0 mg, 0.104 mmol) in dry CH_2Cl_2 (1.5 mL) was added slowly to a stirred solution of DAST (24.0 mg, 0.149 mmol) in dry CH_2Cl_2 (0.5 mL) cooled at -78 °C. After 10 min, the reaction mixture was brought to 20 °C and mixed with cold water (5 mL). The organic layer was separated, washed with water (5 mL), dried over Na_2SO_4, and concentrated *in vacuo* to give a mixture of **32** and **25** as an oil (47.0 mg). IR ($CHCl_3$ cast) 3390 (m), 2960 (m), 1795 (s), 1720 (s), 1695 (s), 1652 (s), 1412 (s), 1365 (s), 1262 (m), 760 (m) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) (minor isomer **32**, 40%) δ 8.08-7.95 (m, 2H, Ar*H*), 7.65-7.25 (m, 8H, Ar*H*), 5.54 (br s, 1H, NC*H*HO), 5.46-5.32 (m, 1H, C*H*OCOPh), 5.25 (d, 1H, J=4 Hz, NC*H*HO), 5.12 (s, 2H, C*H*$_2$Ph), 4.37 (br t, 1H, CbzNC*H*CO), 4.03 (s, 1H, NC*H*N), 3.93 (s, 1H, NC*H*CO), 2.92 (s, 3H, CH_3N), 2.35-1.80 (m, 5H, N*H* and CH_2CH_2), 0.92 (s, 9H, $(CH_3)_3C$); (major isomer **25**, 60%) δ 8.08-7.95 (m, 2H, Ar*H*), 7.65-7.25 (m, 8H, Ar*H*), 5.54 (br s, 1H, NC*H*HO), 5.46-5.32 (m, 1H, C*H*OCOPh), 5.25 (d, 1H, J=4Hz, NC*H*HO), 5.16 (s, 2H, C*H*$_2$Ph), 4.33 (br t, 1H, CBzNC*H*CO), 4.15 (br s, 1H, NC*H*N), 3.74 (br s, 1H, NC*H*CO), 2.87 (s, 3H, C*H*$_3$N), 2.35-1.80 (m, 5H, N*H* and C*H*$_2$C*H*$_2$), 0.97 (s, 9H, (CH$_3$)$_3$C); MS 537.2493 (537.2475 calcd for $C_{29}H_{35}N_3O_7$).

(2R, 6S)-4-Fluoro-2,6-diaminopimelic Acid (33) and (2R, 6S)-4-Hydroxy-2,6-diaminopimelic Acid (34)

Since hydrogen fluoride is highly toxic, the reaction was done in an efficient hood and full protective clothing (face shield, rubber gloves etc.) was used. The (2R, 3S, 6S)-3-hydroxy-2,6-diaminopimelic acid (**26**) (500 mg, 2.43 mmol) was dissolved in anhydrous liquid hydrogen fluoride (10 mL) at -78 °C in a polyethylene vessel equipped with a drying tube. Sulfur tetrafluoride (0.70 mL, 12.3 mmol) was then introduced into the solution and stirring was continued at -78 °C for 10 h. The excess HF and SF$_4$ were removed by a stream of argon gas passed through the solution which was gradually warmed to 20 °C. The toxic effluent gases were absorbed into a cold 3N NaOH solution. The residue was taken up in concentrated HCl and evaporated *in vacuo* to dryness. This process was repeated three times to give a solid (680 mg), one part of which (39.0 mg) was purified by HPLC[117] to afford pure **33** (14.4 mg, 50 % yield) and **34** (14.0 mg, 49%). For **33**: IR (KBr) 3600-2500 (s), 1631 (s), 1507 (m), 1407 (m)cm^{-1}; ^{1}H NMR (200 MHz, D$_2$O) δ 5.17 (br d, 1 H, 50 Hz ($^2J_{HF}$), C*H*F), 3.91 (t, 2 H, 6.5 Hz, 2 OCC*H*N), 2.50-2.02 (m, 4, C*H*$_2$); ^{13}C NMR (75.5 MHz, D$_2$O) δ 174.55 (s, CO), 92.60 (d, 165.3 Hz ($^1J_{CF}$), CF), 52.57 (d, 6.8 Hz ($^3J_{CF}$), CHN), 36.71 (d, 18.9 Hz ($^2J_{CF}$), CH$_2$); ^{19}F NMR (376.5 MHz, D$_2$O) δ -182.57 (s); FAB MS (glycerol) 209 (MH$^+$). For **34**: IR (KBr) 3600-2400 (br, s), 1630 (s), 1507 (m), 1407 (m), 1345 (m) cm^{-1}; ^{1}H NMR (200 MHz, D$_2$O) δ 4.01 (m, 1 H, HOC*H*), 3.93 (dd, 2 H, J_1=6 Hz, J_2 = 7 Hz, 2 OCC*H*N), 2.24-1.95 (m, 4 H, C*H*$_2$CHOHC*H*$_2$); ^{13}C NMR (75.47 MHz, D$_2$O) δ175.05 (s, C=O), 66.86 (s, *C*HOH), 53.24 (s, OC*C*HN), 37.52 (s, *C*H$_2$); FAB-MS 207.10 (MH$^+$) (206.09 calcd for $C_7H_{14}N_2O_5$).

(2S, 3R, 6S)-N, N'-Diacetyl-3-hydroxy-2,6-diaminopimelic Acid (35)

To a solution of β-hydroxydiaminopimelic acid **29** (50 mg, 0.24 mmol) in water (2 mL) at 0 °C was added acetic anhydride (60 μL, 0.64 mmol). The reaction mixture was then stirred at 0 °C for 5h. It was warmed to 20 °C and additional acetic anhydride (2.2 mL) was added portion by portion until the starting material and any ninhydrin active compound had disappeared (checked by TLC). The reaction mixture was then lyophilized *in vacuo* to give an amorphous solid compound (68.0 mg, 96.6%). IR (KBr) 3600-2400 (br, s), 1725 (m), 1632 (s), 1550 (m) cm^{-1}; ^{1}H NMR (360 MHz, D_2O) δ 4.53 (d, 1 H, J=2.8 Hz, HNC*H*CHOH), 4.34 (dd, 1 H, J=4.8 Hz, 8.4 Hz, OCC*H*N), 4.21 (td, 1 H, J=2.8 Hz, 6.4 Hz, HOC*H*), 2.08 (s, 3H, OCCH_3), 2.03 (s, 3 H, OCCH_3), 1.95-1.50 (m, 4 H, CH_2CH_2); FAB-MS 291.26 (MH$^+$) (290.11 calcd for $C_{11}H_{18}N_2O_7$).

Dimethyl (2R, 3S, 6S)-3-Hydroxy-2,6-diaminopimelate (36)

To a solution of β-hydroxydiaminopimelic acid **26** (68.2 mg, 0.331 mmol) in dry MeOH (20 mL) at 0 °C anhydrous HCl gas was added until saturation was reached. The reaction mixture was stirred at 20 °C for 10 h. The solution was concentrated *in vacuo* to give **36** as an amorphous solid (102 mg, 99%). IR (MeOH cast) 3600-2400 (br, s), 1745 (s), 1505 (m), 1442 (m), 1239 (m) cm^{-1}; ^{1}H NMR (200 MHz, D_2O), δ 4.31-4.13 (m, 3 H, OCC*H*N, C*H*OH and NC*H*CHOH), 3.85 (s, 3 H, OCH_3), 3.84 (s, 3 H, OCH_3), 2.26-1.56 (m, 4 H, CH_2CH_2); ^{13}C NMR (75.47 MHz, D_2O) δ 171.25 (s, *C*=O), 169.81 (s, C=O), 58.41 (s, HO*C*HCH$_2$), 54.79 (s, O*C*H$_3$), 54.65 (s, O*C*H$_3$), 53.47 (s, N*C*HCO), 29.68 (s, *C*H$_2$CH$_2$), 27.29 (s, CH$_2$*C*H$_2$); FAB-MS 235.14 (MH$^+$) (235.13 calcd for $C_9H_{19}N_2O_5$); Anal. calcd. for $C_9H_{18}N_2O_5$: C, 46.15; H, 7.75; N 11.96. Found: C, 45.95; H,7.57; N, 11.86.

Dimethyl (2S, 3R, 6S)-3-Hydroxy-2,6-diaminopimelate (36a)

The β-hydroxydiaminopimelic acid **29** (109 mg, 0.529 mmol) was converted to corresponding dimethyl ester by a procedure analogous to the preparation of **36**. The dimethyl ester **36a** was produced in 92% yield (150 mg). IR (MeOH cast) 3600-2400 (br, s), 1745 (s), 1505 (s), 1442 (m), 1239 (m) cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 4.32-4.14 (m, 3 H, OCC*H*N, C*H*OH and NC*H*CHOH), 3.87 (s, 3 H, OCH_3), 3.86 (s, 3 H, OCH_3), 2.29-1.53 (m, 4 H, CH_2CH_2); FAB-MS 235.14 (MH^+) (235.13 calcd for $C_9H_{19}N_2O_5$).

(2R, 3S, 6S)-N,N'-Diacetyl-3-hydroxy-2,6-diaminopimelic Acid (37)

The β-hydroxydiaminopimelic acid **26** (55.1 mg, 0.267 mmol) was converted to corresponding N,N'-diacetyl derivative **37** (72.5 mg, yield: 93.5%)using a procedure analogous to the preparation of **35**. IR (KBr) 3600-2400 (br, s), 1729 (s), 1633 (s), 1549 (s) cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 4.43 (d, 1 H, J=2.8 Hz, HNC*H*CHOH), 4.32 (dd, 1 H, J_1=4.8 Hz, J_2=8.4 Hz, OCC*H*N), 4.08 (td, 1 H, J_1=2.8 Hz, J_2=6.5 Hz, HOC*H*), 2.04 (s, 3 H, $OCCH_3$), 2.00 (s, 3 H, $OCCH_3$), 2.00-1.45 (m, 4 H, CH_2CH_2); MS 272 (M^+ - H_2O) (272.10 calcd for $C_{11}H_{16}N_2O_6$); Anal. calcd. for $C_{11}H_{18}N_2O_7$: C, 45.52; H, 6.25; N, 9.65. Found: C, 45.43; H, 6.17; N, 9.57.

Dimethyl (2R, 3S, 6S)-N, N'-Diacetyl-3-hydroxy-2,6-diaminopimelate (38) and Dimethyl (5S)-N, N'-Diacetyl-2,6-diamino-2-hepten-1,7-dioate (39)

To a solution of **37** (66.0 mg, 0.217 mmol) in MeOH (2 mL) was added dropwise a freshly prepared ethereal solution of CH_2N_2 until the yellow color persisted. The excess CH_2N_2 was removed by blowing Ar through the solution. The reaction mixture was concentrated *in vacuo* to give an oil, which was purified by flash chromatography using acetone to afford pure **38** in 70% yield (51.0 mg) and pure **39** in 7% yield (4.8 mg). For **38**: IR ($CHCl_3$ cast) 3300 (br, s), 2950 (m), 1741 (s), 1656 (s), 1538 (s), 1435 (m), 1375 (m), 1213 (m) cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 4.52 (d, 1 H, J=2.6 Hz, HNC*H*CHOH), 4.40 (dd, 1 H, J_1=5.2 Hz, J_2=8.8 Hz, OCC*H*N), 4.07 (td, 1 H, J_1=2.6 Hz, J_2=6.8 Hz, HOC*H*), 3.75 (s, 3 H, OCH_3), 3.72 (s, 3 H, OCH_3), 2.04 (s, 3 H, $OCCH_3$), 1.99 (s, 3 H, $OCCH_3$), 1.98-1.45 (m, 4 H, CH_2CH_2); MS 300.1315 (M^+ - H_2O) (300.1321 calcd for $C_{13}H_{20}N_2O_6$); Anal. calcd for $C_{13}H_{22}N_2O_7$: C, 49.06; H, 6.92; N, 8.81. Found: C, 48.91; H, 7.19; N, 8.92.

For **39**: IR ($CHCl_3$ cast) 3200 (br, s), 2940 (m), 1735 (s), 1650 (s), 1540 (s), 1437 (m), 1373 (m), 1215 (m) cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 6.64 (t, 1H, J=7.2 Hz, C=C*H*), 4.43 (dd, 1H, J=4.8, 10 Hz, NC*H*CO), 3.77 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 2.36-1.72 (m, 4H, CH_2CH_2), 2.09 (s, 3H, $OCCH_3$), 2.00 (s, 3H, $OCCH_3$); MS 300.1313 (300.1321 calcd for $C_{13}H_{20}N_2O_6$).

(4S)-2,5-Dioxo-4-(1-methylethyl)oxazolidine (40)

The literature procedure was modified.[164] A stream of phosgene was introduced to a stirred suspension of L-valine (46.8 g, 0.400 mol) in anhydrous THF (600 mL) for 1.5 h until solution was complete. A stream of Ar was bubbled through the mixture to remove excess phosgene. The solvent was removed *in vacuo* (40 °C). The residue was dissolved in dry THF and solvent was evaporated *in vacuo* to remove HCl. The process was repeated twice and a white solid product was obtained in quantitative yield (57.3 g). mp 80 °C (lit.[149] 80-81 °C); IR ($CHCl_3$ cast) 3296 (s), 2920 (m), 1840 (s), 1814 (s), 1755 (s), 1370 (s), 1273 (s), 943 (s) cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 7.20 (s, 1 H, *H*NCO), 4.25 (d, 1 H, J = 4 Hz, HNC*H*CO), 2.25 (m, 1 H, CH_3CHCH_3), 1.10 (d, 3 H, J = 5 Hz, CH_3), 1.00 (d, 3 H, J = 5 Hz, CH_3); MS 143.0581 (143.0582 calcd for $C_6H_9NO_3$).

(3S)-3-(1-Methylethyl)-2,5-piperazindione (41)

The literature procedure was modified.[164] To a stirred solution of ethyl glycinate hydrochloride (25.5 g, 0.182 mol) in dry CH_2Cl_2 (400 mL) at 0 °C, was added dropwise freshly distilled Et_3N. The resultant solution was cooled to -78 °C and a solution of **40**

(25.7g, 0.180 mol) in THF (200 mL) precooled at -78 °C was added dropwise. The mixture was stirred at - 78 °C for 3 h and warmed to 20 °C for an additional 2.5 h. $Et_3N{\cdot}HCl$ was filtered and the filtrate was concentrated *in vacuo* to give **41a**, which was dissolved in dry toluene (1010 mL). The solution was heated to reflux overnight with stirring and then cooled to 0 °C. A white precipitate of **41** was filtered and washed with Et_2O (3 x 300 mL) (18.2 g, 29% yield). IR ($CHCl_3$ cast) 3200-2860 (m), 1665 (s), 1460 (m), 1350 (m), 800 (m) cm^{-1}; ^1H NMR (80 MHz, DMSO-d_4) δ 3.75 (d, 1 H, J=6 Hz, i-PrC*H*CONH), 3.58 (s, 2 H, HNCH_2CO), 2.10 (m, 1 H, CH_3C*H*CH_3), 0.93 (d, 3 H, J=6 Hz, CH_3), 0.83 (d, 3 H, J=6 Hz, CH_3); MS 156.0898 (156.0898 calcd for $C_7H_{12}N_2O_2$). Anal. calcd. for $C_7H_{12}N_2O_2$: C, 53.83; H, 7.74; N, 17.94. Found: C, 53.54; H, 7.60; N, 17.77.

(3S)-3,6-Dihydro-3-(1-methylethyl)-2,5-dimethoxypyrazine (42)

The literature procedure was modified.[164] To a suspension of **41**(1.00 g, 6.41 mmol) in CH_2Cl_2 (60 mL) at 20 °C, was added trimethyloxonium tetrafluoroborate (2.30 g, 17.5 mmol). The reaction mixture was then vigorously stirred for 3 days at 20 °C (after 24 h, an additional 0.92 g $(Me_3O)BF_4$ was added). After addition of a solution of $NaH_2PO_4{\cdot}H_2O$ (3.48 g, 25.2 mmol) and $Na_2HPO_4{\cdot}7H_2O$ (22.31 g, 83.2 mmol) in H_2O (70 mL), the organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo* to give a liquid, which was distilled under vacuum (80 °C / 5mmHg) to give **42** (1.12 g, 95% yield). $[\alpha]_D$ +103.3 (c 0.85, 95% EtOH); (lit.[164] $[\alpha]_D$ +106.3, c 1.0, EtOH); IR ($CHCl_3$ cast) 2950 (s), 1702 (s), 1463 (m), 1437 (s), 1312 (m), 1246 (s), 1196 (m), 1104 (m), 1010 (s), 759 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.01 (s, 3 H, iPrC*H*N and CH_2N), 3.74 (s, 3 H, CH_3O), 3.70 (s, 3 H, CH_3O), 2.25 (m, 1 H, CH_3C*H*CH_3), 1.05 (d, 3 H, J=7 Hz, CH_3C), 0.77 (d, 3 H, J=7.5 Hz, CH_3C); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ 164.87 (s, OC=N), 162.35 (s, O=CN), 61.09 (s, iPrCH), 52.51

(s, OCH_3), 52.47 (s, OCH_3), 46.63 (s, NCH_2), 32.47 (s, Me*C*HMe), 19.04 (s, *C*H_3C), 17.02 (s, *C*H_3C); MS 184.1213 (184.1212 calcd for $C_9H_{16}N_2O_2$); Anal. calcd for $C_9H_{16}N_2O_2$: C, 58.69; H, 8.76; N, 15.22. Found: C, 58.76; H, 8.91; N, 15.34.

(4S, 3'S, 3"S, 6"R)-4-[(3,6-Dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazin-6-yl)-3-hydroxypropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (43) and (4S, 3'R, 3"S, 6"R)-4-[(3,6-Dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazin-6-yl)-3-hydroxypropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (44)

To a solution of (3S)-3,6-dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazine (**42**) (0.287 g, 1.56 mmol) in dry THF (8 mL) at -78 °C was added dropwise a solution of *n*-BuLi in hexane (1.23 N, 1.27 mL, 1.56 mmol). The mixture was stirred at -78 °C for 35 min. Aldehyde **15** (0.432 g, 1.56 mmol) in dry THF (12 mL) was added dropwise to the yellow basic solution which turned colorless. The mixture was stirred at -78 °C for another 7 h, quenched at -78 °C with HOAc (0.19 g , 3.17 mmol) in THF (3 mL), and warmed to 20 °C. The solvent was removed *in vacuo*, and the residue was partitioned between water and dichloromethane, and the aqueous phase was extracted with dichloromethane (2 x 30 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give an oil which was purified by flash chromatography (ethyl actetate:petroleum ether, 50:50) to afford an inseparable mixture of **43** (3'S isomer) and **44** (3'R isomer) (83:17 ratio) (total 0.376 g, 52 % yield): IR ($CHCl_3$ cast) 3460 (m), 2940 (m), 1800 (m), 1700 (br s), 1415 (m), 1238 (m), 765 (m), 700 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 50 °C) δ (major isomer **43**, 83%) 7.35 (m, 5 H, Ar*H*), 5.53 and 5.24 (AB, 2 H, 6 Hz, CbzNC*H*$_2$O), 5.22 and 5.20 (AB, 2 H, C*H*$_2$Ph), 4.40 (t, 1 H, 6 Hz, OCC*H*NCbz), 3.99 (t, 1 H, 4 Hz, i-Pr-C*H*), 3.96 (br, 1 H, *H*COH), 3.90 (t, 1 H, 4 Hz, NC*H*COMe), 3.74 (s, 3 H, OC*H*$_3$), 3.69 (s, 3 H, OC*H*$_3$), 2.25 (m, 1 H, C*H*Me$_2$), 2.03 (br d, 1 H, 7 Hz, O*H*), 2.25-2.05 (m, 2 H, C*H*$_2$CH$_2$), 1.85-1.65 (m, 2 H, CH$_2$C*H*$_2$), 1.05 (d, 3 H, 8

Hz, $\mathrm{C}H_3$), 0.74 (d, 3 H, 8 Hz, $\mathrm{C}H_3$); (minor isomer **44**, 17%) 7.35 (m, 5 H, Ar*H*), 5.51 and 5.16 (AB, 2 H, $\mathrm{CbzNC}H_2\mathrm{O}$), 5.22 and 5.20 (AB, 2 H, $\mathrm{C}H_2\mathrm{Ph}$), 4.33 (t, 1 H, 7Hz, OCC*H*NCbz), 4.12 (t, 1 H, 4.8 Hz, *i*-Pr-C*H*), 3.96 (br, 1 H, *H*COH), 3.70 (s, 3 H, $\mathrm{OC}H_3$), 3.68 (s, 3 H, $\mathrm{OC}H_3$), 2.81 (d, 1 H, O*H*), 2.25 (m, 1 H, $\mathrm{C}H\mathrm{Me}_2$), 2.25-2.05 (m, 2 H, $\mathrm{C}H_2\mathrm{CH}_2$), 1.85-1.65 (m, 2 H, $\mathrm{CH}_2\mathrm{C}H_2$), 1.04 (d, 3 H, 8 Hz, $\mathrm{C}H_3$), 0.74 (d, 3 H, 8 Hz, $\mathrm{C}H_3$); ^{13}C NMR (75.5 Hz, D_2O) δ 172.3, 166.0, 161.7, 151.51, 135.5, 128.7, 128.6, 128.3, 78.2, 72.0, 68.0, 61.1, 60.4, 59.4, 54.9, 52.7, 32.1, 28.6, 19.0, 16.9; MS 461.2166 (461.2162 calcd for $C_{23}H_{31}N_3O_7$). Anal. calcd for $C_{23}H_{31}N_3O_7$: C, 59.86; H, 6.77; N, 9.11. Found: C, 59.75; H, 6.89; N, 8.95.

Hydrolysis of the Mixture of 43 and 44 to (2R, 3S, 6S)-3-Hydroxy-2,6-diaminopimelic Acid (26) and (2R, 3R, 6S)-3-Hydroxy-2,6-diaminopimelic Acid (45)

Deprotection of the mixture of **43** and **44** (23.8 mg, 51.6 mmol) in 6N HCl (10 mL) by a procedure analogous to that described below for hydrolysis of **48** to **56** produced the mixture of **43** and **44** as free amino acids in quantitative yield. The sample showed identical chromatographic and spectral properties to **26** except for: ^{1}H NMR (300 MHz, D_2O, DSS) δ (major isomer 83 %, peaks enhanced if pure **26** added) 4.13-4.01 (m, 1 H, C*H*OH), 3.79 (t, 1 H, 6 Hz, $\mathrm{CH}_2\mathrm{C}H\mathrm{N}$), 3.65 (d, 1 H, 5.2 Hz, C*H*NCHOH), 2.20-1.54 (m, 4 H, $\mathrm{C}H_2\mathrm{C}H_2$); (minor isomer **45**, 17 %) 4.13-4.01 (m, 1 H, C*H*OH), 3.79 (t, 1 H, 6 Hz, $\mathrm{CH}_2\mathrm{C}H\mathrm{N}$), 3.66 (d, 1 H, 4.8 Hz, C*H*NCHOH), 2.20-1.54 (m, 4 H, $\mathrm{C}H_2\mathrm{C}H_2$); ^{13}C NMR (75.5 Hz, D_2O) δ (major isomer 83%, peaks enhanced if pure **26** added) 175.14, 173.42, 69.93, 60.06, 55.17, 30.06, 27.85; (minor isomer **45**, 17%) 175.14, 173.42, 70.08, 60.01, 55.17, 30.01. 27.85.

(4S, 3'R, 3"R, 6"S)-4-[(3,6-Dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazin-6-yl)-3-hydroxypropyl]-3-(benzyloxycarbonyl)-5-

oxazolidinone (46) and (4S, 3'S, 3"R, 6"S)-4-[(3,6-Dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazin-6-yl)-3-hydroxypropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (47)

These compounds were made as a mixture by following the procedure described above for **43** and **44** using (3R)-3,6-dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazine (0.361 g, 1.96 mmol) and **15** (0.544 g, 1.96 mmol). The purified product was an inseparable mixture of **46** (3'R isomer) and **47** (3'S isomer) (55:45 ratio) (total 0.318 g, 35 % yield): IR ($CHCl_3$ cast) 3350 (br), 2960 (m), 1802 (s), 1700 (s), 1416 (s), 1240 (s), 765 (m), 700 (m) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ (major diastereomer **46**, 55%) 7.30 (m, 5 H, Ar*H*), 5.50 (m, 1 H, CbzNCH*H*O), 5.18 (m, 1 H, CbzNCH*H*O), 5.15 (s, 2 H, CH_2Ph), 4.34 (t, 1 H, 7 Hz, OCC*H*NCbz), 3.96 (m, 1 H, i-PrC*H*), 3.93 (br, 1 H, *H*COH), 3.86 (m, 1 H, NC*H*COMe), 3.69 (s, 3 H, OCH_3), 3.64 (s, 3 H, OCH_3), 2.22 (m, 2 H, CH_2CH$_2$), 1.02 (d, 3 H, 8 Hz, CH_3), 0.70 (d, 3 H, 8 Hz, CH_3); (minor diastereomer **47**, 45%) 7.30 (m, 5 H, Ar*H*), 5.50 (m, 1 H, CbzNCH*H*O), 5.18 (m, 1 H, CbzNCH*H*O), 5.15 (s, 2 H, CH_2Ph), 4.39 (t, 1 H, 6 Hz, OCC*H*NCbz), 3.96 (m, 1 H, i-PrC*H*), 3.93 (br, 1 H, *H*COH), 3.86 (m, 1 H, NC*H*COMe), 3.66 (s, 3 H, OCH_3), 3.64 (s, 3 H, OCH_3), 2.22 (m, 1 H, MeC*H*Me), 2.33-1.86 (m, 3 H, O*H* and CH_2CH$_2$), 1.86-1.46 (m, 2 H, CH$_2$CH_2), 1.01 (d, 3 H, 8 Hz, CH_3), 0.70 (d, 3 H, 8 Hz, CH_3); MS 461.2153 (461.2162 calcd for $C_{23}H_{31}N_3O_7$). Anal. calcd for $C_{23}H_{31}N_3O_7$: C, 59.86; H, 6.77; N, 9.11. Found: C, 59.83; H, 6.86; N, 8.75.

(4S, 3'R, 3"S, 6"S)-4-[(3,6-Dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazin-6-yl)-3-fluoropropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (48) and (4S, 3'S, 3"S, 6"S)-4-[(3,6-Dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazin-6-yl)-3-fluoropropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (49)

A solution of a mixture **43** and **44** (0.280 g, 0.61 mmol) in dry dichloromethane (5 mL) was added dropwise to a stirred solution of (diethylamino)sulfur trifluoride (DAST) (0.16 mL, 1.21 mmol) in dry dichloromethane (5 mL) at -78 °C, and this was stirred at -78 °C for an additional 2 h. Aqueous $NaHCO_3$ solution (0.20 g / 20 mL water) was added, the mixture was warmed to 20 °C, and the layers were separated. The aqueous layer was extracted with dichloromethane (3 x 20 mL), and the combined organic phases were dried (Na_2SO_4) and concentrated *in vacuo* to afford an oil (0.280 g), which was separated by MPLC (silica gel column, 2.3 x 30 cm; ethyl acetate:hexane, 25:75). The fractions were monitored by TLC with detection by phosphomolybdic acid / cerium sulfate spray. Repeated chromatography was necessary to obtain pure **48** (42 mg, 15 % yield) and impure **49** which was hydrolyzed directly to **57** (see below). Along with **48** and **49**, byproducts **50** (27 mg, 10% yield) and **51** (81 mg, 30% yield) were also isolated; and their spectroscopic data are given below. For **48**: IR ($CHCl_3$ cast) 2960 (br), 1803 (s), 1719 (s), 1705 (s), 1668 (s), 1649 (m), 1416 (br), 1360 (s), 1242 (s), 1051 (m), 765 (m), 699 (m) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.36 (s, 5 H, Ar*H*), 5.56 and 5.22 (AB, 2 H, J=4 Hz, $\mathrm{CbzNC}\mathit{H}_2\mathrm{O}$), 5.19 (s, 2 H, $\mathrm{C}\mathit{H}_2\mathrm{Ph}$), 4.82 (br d, 1 H, 48 Hz ($^2J_{HF}$), C*H*F), 4.34 (t, 1 H, CbzNC*H*CO), 4.27 (d of t, 1 H, 20 Hz ($^3J_{HF}$), 4 Hz, NC*H*COMe), 3.94 (t, 1 H, 4 Hz, iPrC*H*N), 3.72 (s, 3H, $\mathrm{O}\mathrm{C}\mathit{H}_3$), 3.71 (s, 3H, $\mathrm{O}\mathrm{C}\mathit{H}_3$), 2.30 (m, 1 H, MeC*H*Me), 2.25-1.86 (m, 4 H, $\mathrm{C}\mathit{H}_2\mathrm{C}\mathit{H}_2$), 1.06 (d, 3 H, 7.2 Hz, $\mathrm{C}\mathit{H}_3$), 0.71 (d, 3 H, 7.2 Hz, $\mathrm{C}\mathit{H}_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 171.86, 165.79, 160.09, 153.00, 135.38, 128.79, 128.70, 128.33, 93.49 (178.3 Hz, $^1J_{CF}$), 68.12, 60.76, 58.84, 58.56, 54.52, 52.79 (9.3 Hz, $^2J_{CF}$), 31.51, 25.87, 19.16, 16.65; ^{19}F NMR (376.5 MHz, $CDCl_3$) (^{1}H decoupled) Two lines with equal intensities: δ -190.48 and -190.63; MS 463.2132 (463.2118 calcd for $C_{23}H_{30}N_3O_6F$). Anal. calcd for $C_{23}H_{30}N_3O_6F$: C, 59.60; H, 6.53; N, 9.07. Found: C, 59.93; H, 6.54; N, 8.60.

(4S)-4-[3-(2,5-Dimethoxy-3-(1-methylethyl)pyrazin-6-yl)propyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (50)

Compound **50** was produced from two different reactions:

1) It was isolated from the DAST reaction of **43** and **44** described above in 10% yield (27 mg)

2) To a stirred solution of N-chlorosuccinimide (54.8 mg, 0.41 mmol) in anhydrous THF (3 mL) was added dropwise a solution of Ph_3P (107 mg, 0.41 mmol) in anhydrous THF (4 mL). To this suspension was added a mixture of **43** and **44** (172 mg, 0.373 mmol) in dry THF (10 mL). After being stirred at 20 °C overnight, the reaction mixture was poured into Et_2O / CH_2Cl_2 (1:1, 20 mL). It was dried over Na_2SO_4 and concentrated *in vacuo* to give an oil, which was purified by flash chromatography using EtOAc : Hexane (25 : 75), to give **50** in 13% yield (22 mg). IR ($CHCl_3$ cast) 2965 (s), 1805 (s), 1720 (s), 1462 (s), 1416 (s), 1374 (s), 1354 (s), 1202 (s), 1168 (s), 1127 (s), 1048 (s), 1025 (s), 765 (m), 700 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.35 (s, 5 H, ArH), 5.56 (br d, 1 H, NC*H*O), 5.24 (m, 1 H, NC*H*O),5.19 (s, 2 H, CH_2Ph), 4.40 (t, 1 H, J=5 Hz, NC*H*CO), 3.90 (s, 3 H, OCH_3), 3.88 (s, 3 H, OCH_3), 3.25 (p, 1 H, J=6.4 Hz, MeC*H*Me), 2.70 (t, 2 H, J=6.6 Hz, NCCH_2), 2.20-1.67 (m, 4 H, CH_2CH_2), 1.24 (s, 3 H, CH_3CH), 1.20 (s, 3 H, CHCH_3); MS 443.2033 (443.2056 calcd for $C_{23}H_{29}N_3O_6$).

(4S,3'S)-4-[3-(3-Hydro-2,5-dimethoxy-3-(1-methylethyl)pyrazin-6-yl)-3-propylidene]-3-(benzyloxycarbonyl)-5-oxazolidinone (51)

Compound **51** was isolated from the DAST reaction of **43** and **44** described above in 30% yield (81 mg). IR ($CHCl_3$ cast) 2960 (s), 1803 (s), 1718 (s), 1667 (s), 1646 (s), 1438 (s), 1414 (s), 1359 (s), 1242 (s), 1127 (s), 1051 (s), 1024 (s), 765 (m), 698 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.38 (s, 5 H, Ar*H*), 5.82 (t, 1 H, J=7.2 Hz, CC*H*CH$_2$), 5.52 (s, 1 H, NC*H*O), 5.18 (m, 1 H, NC*H*O), 5.15 (s, 2 H, CH_2Ph), 4.34 (br t, 1 H, NC*H*CO), 4.12 (d, 1 H, J=2.4 Hz, *i*PrC*H*N), 3.70 (s, 3 H, OCH_3), 3.69 (s, 3 H, OCH_3), 2.66-1.92 (m, 5 H, CH_2CH_2 and MeC*H*Me), 0.98 (d, 3 H, J = 7.2 Hz, CH_3CH), 0.64 (d, 3 H, J=7.2 Hz, CHCH_3); MS 443.2049 (443.2056 calcd for $C_{23}H_{29}N_3O_6$).

(4S, 3'S, 3"R, 6"R)-4-[(3,6-Dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazin-6-yl)-3-fluoropropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (52) and (4S, 3'R, 3"R, 6"R)-4-[(3,6-Dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazin-6-yl)-3-fluoropropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (53)

Compound **52** was prepared by the procedure described above for **48** using the mixture of **46** and **47** (217 mg, 0.470 mmol) and (diethylamino)sulfur trifluoride (146 mg, 0.910 mmol). Repeated chromatographic separation afforded pure **52** (18.0 mg, 8.3 % yield) and impure **53** which was hydrolyzed directly to **55** (see below). For **52**: IR ($CHCl_3$ cast) 2960 (m), 1802 (s), 1717 (s), 1700 (s), 1415 (m), 1241 (s), 765 (m) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.36 (s, 5 H, Ar*H*), 5.54 (br s, 1 H, CbzNCH*H*O), 5.24-5.16 (m, 3 H, CbzNCH*H*O and CH_2Ph), 4.79 (br d, 1 H, 48 Hz ($^2J_{HF}$), C*H*F), 4.33 (t, 1 H, 6 Hz, CbzNC*H*CO), 4.25 (d of t, 1 H, 2.8 Hz ($^3J_{HH}$), 22 Hz ($^3J_{HF}$), NC*H*COMe), 3.92 (t, 1 H, 3 Hz, iPrCHN), 3.71 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 2.28 (m, 1 H, MeC*H*Me), 2.21-1.63 (m, 4 H, CH_2CH_2), 1.08 (d, 3 H, 8 Hz, CH_3) and 0.71 (d, 3 H, 8 Hz, CH_3); ^{19}F NMR (376.5 MHz, $CDCl_3$) (^{1}H decoupled) Two lines with equal intensities; δ -190.99, -191.09; MS 463.2126 (463.2118 calcd for $C_{23}H_{30}N_3O_6F$). Anal. calcd for $C_{23}H_{30}N_3O_6F$: C, 59.60; H, 6.53; N, 9.07. Found: C, 60.06; H, 6.66; N, 8.68.

(2R, 3S, 6S)-3-Fluoro-2,6-diaminopimelic Acid (54)

The protecting groups were removed from **52** (32.0 mg, 69.1 µmol) by a hydrolysis procedure analogous to that described below for production of compound **56** to give (after chromatography on cellulose) pure **54** (9.2 mg, 47% yield) : IR (MeOH cast) 3420 (br, m), 2920 (br, s), 1666 (s), 1580 (m), 1487 (s) cm^{-1}; ^{1}H NMR (200 MHz, D_2O, DSS) δ 4.96 (br d, 1 H, 48 Hz ($^2J_{HF}$), $C\mathit{H}F$), 4.12 (dd, 1 H, 24.6 Hz ($^3J_{HF}$), 2.6 Hz ($^3J_{HH}$), $CFC\mathit{H}N$), 3.95 (t, 1 H, 6 Hz, $CH_2C\mathit{H}N$), 2.20-1.58 (m, 4 H, $C\mathit{H}_2C\mathit{H}_2$); ^{13}C NMR (75.5 MHz, D_2O) δ 174.74 (s, CO), 170.64 (s, CO), 92.40 (d, 175.2 Hz ($^1J_{CF}$), CF), 58.0 (d, 26.4 Hz ($^2J_{CF}$), $N\mathit{C}HCF$), 54.90 (s, $CH_2\mathit{C}HN$), 27.35 (s, $CH_2\mathit{C}H_2$), 26.65 (d, 21.71 Hz ($^2J_{CF}$), $\mathit{C}H_2CH_2$); ^{19}F NMR (376.5 MHz, D_2O) δ -190.88 (s); FAB MS (glycerol) 209.14 (MH$^+$).

(2R, 3R, 6S)-3-Fluoro-2,6-diaminopimelic Acid (55)

The fraction (60.0 mg) from MPLC purification containing crude **53** was hydrolyzed by a procedure analogous to that described below for production of **56** from **48**. Repeated purification by ion exchange chromatography on AG50 (H$^+$ form, 100 mesh, HCl gradient) and cellulose chromatography (as described for **56**) afforded pure **55** (6.8 mg, 2.3% overall yield for DAST fluorination and hydrolysis): IR (MeOH cast) 3600-2600 (br s), 1734 (m), 1653 (s), 1580 (m), 1496 (m), 1405 (m) cm^{-1}; ^{1}H NMR (200 MHz, D_2O, DSS) δ 5.02 (br d, 1 H, 47.2 Hz ($^2J_{HF}$), $C\mathit{H}F$), 4.03 (dd, 1 H, 24.0 Hz ($^3J_{HF}$), 4.8 Hz, $CFC\mathit{H}N$), 3.97 (t, 1 H, 6 H z, $CH_2C\mathit{H}N$), 2.20-1.70 (m, 4 H, $C\mathit{H}_2C\mathit{H}_2$); ^{13}C NMR (75.5 MHz, D_2O) δ 173.47 (s, CO), 170.83 (s, CO), 92.15 (d, 175.2 Hz ($^1J_{CF}$), CF), 57.80 (d, 22.6 Hz ($^2J_{CF}$), $\mathit{C}HNCHF$), 53.92 (s, CH_2CHN), 28.10 (d, 20.6 Hz ($^2J_{CF}$), $CHF\mathit{C}H_2$), 26.70 (s, $CH_2\mathit{C}H_2$); ^{19}F NMR (376.5 MHz, D_2O) δ -192.22 (s); FAB MS (glycerol) 209 (MH$^+$).

(2S, 3R, 6S)-3-Fluoro-2,6-diaminopimelic Acid (56)

The protecting groups were removed by aqueous hydrolysis. Compound **48** (36.0 mg, 77.8 μmol) was heated to reflux in 6N HCl (10 mL) for 6 h. The aqueous solution was then washed with ethyl ether (2 x 20 mL) and concentrated *in vacuo* to give a solid, which was purified by column chromatography on cellulose powder (2.5 x 26 cm, solvent system: *n*-BuOH / H_2O / CH_3CO_2H, 4 / 2 / 1) with slight pressure of argon or nitrogen. Since β-fluorodiaminopimelates are unstable under basic conditions, compound **56** was obtained as its bis-hydrochloride by lyophilization from dilute hydrochloric acid to give 11.2 mg (51 % yield) : IR (KBr) 3600-2600 (br, s), 1735 (m), 1630 (m), 1505 (m), 1400 (m), ^1H NMR (200 MHz, D_2O, DSS) δ 4.95 (br d, 1 H, 46.4 Hz ($^2J_{HF}$), C*H*F), 4.12 (dd, 1 H, 24.6 Hz ($^3J_{HF}$), 2.6 Hz, FCC*H*N), 3.94 (t, 1 H, 6 Hz, $CH_2C\mathit{H}N$), 2.20-1.60 (m, 4 H, $C\mathit{H}_2C\mathit{H}_2$); ^{13}C NMR (75.5 MHz, D_2O) δ 172.90 (s, CO), 169.63 (s, CO), 92.12 (d, 175.8 Hz ($^1J_{CF}$), CF), 57.63 (br s, CF*C*N), 53.60 (br s, $CH_2\mathit{C}HN$), 26.94 (s, $CH_2\mathit{C}H_2CHN$), 26.67 (d, 18.9 Hz ($^2J_{CF}$), $CHF\mathit{C}H_2$); ^{19}F NMR (376.5 MHz, D_2O) δ -191.38; FAB MS (glycerol) 209.13 (MH^+).

(2S, 3S, 6S)-3-Fluoro-2,6-diaminopimelic Acid (57)

The fraction (93 mg) from MPLC purification containing crude **49** was hydrolyzed by a procedure analogous to that described for production of **56** from **48**. Repeated purification by chromatography on cellulose and ion exchange chromatography on AG50 (H^+ form, 1 mL bed volume, 6N HCl) afforded **57** (2.4 mg, 0.8 % overall yield for DAST fluorination and hydrolysis): IR (KBr) 3600-2600 (br, s), 1718 (m), 1657 (s), 1593 (m), 1490 (s), 1399 (m), 1150 (m) cm^{-1}; ^1H NMR (200 MHz, D_2O, DSS) δ 4.92 (br d, 1 H, 48.0 Hz ($^2J_{HF}$), C*H*F), 3.79 (dd, 1 H, 24.3 Hz ($^3J_{HF}$), 4.4 Hz, FCC*H*N), 3.69 (t, 1 H, 6 Hz, $CH_2C\mathit{H}N$), 2.10-1.65 (m, 4 H, $C\mathit{H}_2C\mathit{H}_2$); ^{19}F NMR (376.5 MHz, D_2O) δ -191.83; FAB MS (glycerol) 209.11 (MH^+).

(3S)-[6,6-^{2}H]-3,6-Dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazine (58)

To a solution of (3S)-3,6-dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazine (**42**) (1.55 g, 8.43 mmol) in dry THF (20 mL) at -78 °C was added dropwise a solution of *n*-BuLi (2.40 M, 3.86 mL, 1.1 eq). Stirring was continued at -78 °C for 40 min, at which point the yellow solution was quenched with CD_3OD (0.334 g, 1.1 eq). The sequence of anion formation and quenching was then repeated twice with 1.2 eq and 1.3 eq *n*-BuLi, respectively. After an additional 40 min at -78 °C , the reaction was further quenched with D_2O (2 mL). The solution was partitioned between dichloromethane (30 mL) and water (30 mL), and the combined organic phases were dried (Na_2SO_4) and concentrated *in vacuo*. The resulting liquid was distilled under reduced pressure (70 °C / 2 torr) to afford **58** as a colorless liquid (1.21 g, 77 % yield): IR ($CHCl_3$ cast) 2980 (s), 1695 (s), 1436 (m), 1302 (s), 1237 (s), 1139 (s), 1031 (m), 745 (s) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.00 (d, 1 H, 4 Hz, iPrC*H*N), 3.74 (s, 3 H, OC*H*3), 3.71 (s, 3 H, OC*H*3), 2.24 (m, 1 H, MeC*H*Me), 1.03 (d, 3 H, 7 Hz, C*H*3C), 0.76 (d, 3 H, 7 Hz, C*H*3C); MS 186.1334 (186.1335 calcd for $C_9H_{14}D_2N_2O_2$). No peak for $C_9H_{15}DN_2O_2$. Anal. calcd for $C_9H_{14}D_2N_2O_2$: C, 58.06; H, 8.72; N, 15.05. Found: C, 58.27; H, 8.95; N, 14.97.

(4S, 3'S, 3"S, 6"R)-4-[(3-^{2}H-3-hydro-2,5-dimethoxy-3-(1-methylethyl)pyrazin-6-yl)-3-hydroxypropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (59) and (4S, 3'R, 3"S, 6"S)-4-[(3-^{2}H-3-hydro-2,5-dimethoxy-3-(1-methylethyl)pyrazin-6-yl)-3-fluoropropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (60) and (2S, 3R, 6S)-[2-^{2}H]-3-Fluoro-2,6-diaminopimelic Acid (61)

The aldol condensation between the deuterated bislactim ether **58** (1.09 g, 5.85 mmol) and aldehyde **15** (1.62 g, 5.85 mmol) was accomplished by a procedure analogous to that described for generation of the mixture of 43 and 44. After purification as described before, 0.948 g (35 % yield) of (4S, 3'RS, 3"S, 6"R)-[6"-^{2}H]-4-[(3,6-dihydro-2,5-

dimethoxy-3-(1-methylethyl)pyrazin-6-yl)-3-hydroxypropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (**59**) was obtained: IR (CHCl$_3$ cast) 3480 (br m), 2959 (s), 1803 (s), 1699 (s), 1419 (s), 1240 (s), 1128 (m), 1010 (m), 765 (m) cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$, 50 °C) δ (major diastereomer **59**) 7.35 (m, 5 H, Ar*H*), 5.53 and 5.24 (AB, 2 H, 6 Hz, CbzNCH_2O), 5.22 and 5.20 (AB, 2 H, CH_2Ph), 4.40 (t, 1 H, 6 Hz, OCC*H*NCbz), 4.00 (d, 1 H, 4 Hz, iPrC*H*), 3.74 (s, 3 H, OCH_3), 3.69 (s, 3 H, OCH_3), 2.25 (m, 1 H, MeC*H*Me), 2.25-2.05 (m, 2 H, CH_2CH$_2$), 1.85-1.65 (m, 2 H, CH$_2$CH_2), 1.76 (br, 1 H, O*H*), 1.05 (s, 3 H, 8 Hz, CH_3C), 0.74 (s, 3 H, 8 Hz, CH_3C); (minor diastereomer) 7.35 (m, 5 H, Ar*H*), 5.51 and 5.16 (AB, 2 H, 3 Hz, CbzNCH_2O), 5.22 and 5.20 (AB, 2 H, CH_2Ph), 4.33 (t, 1 H, 7 Hz, OCC*H*NCbz), 4.12 (d, 1 H, 4 Hz, iPrC*H*), 3.70 (s, 3 H, OCH_3), 3.68 (s, 3 H, OCH_3), 2.90 (d, 1 H, O*H*), 2.25 (m, 1 H, MeC*H*Me), 2.25-2.05 (m, 2 H, CH_2CH$_2$), 1.85-1.65 (m, 2 H, CH$_2$CH_2), 1.04 (d, 3 H, 8 Hz, CH_3), 0.74 (d, 3 H, 8 Hz, CH_3); CI MS 463 (MH$^+$). Anal. calcd for C$_{23}$H$_{30}$DN$_3$O$_7$: C, 59.80; H, 6.76; N, 9.09. Found: C, 59.99; H , 6.75; N, 8.87.

The fluorination of **59** (0.892 g, 1.93 mmol) with DAST employed a procedure analogous to that described for generation of **48** to produce 90.2 mg (10 % yield) of (4S, 3'R, 3"S, 6"S)-[6-^{2}H]-4-[(3,6-dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazin-6-yl)-3-fluoropropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (**60**): IR (CHCl$_3$ cast) 2960 (s), 1803 (s), 1718 (s), 1668 (s), 1647 (m), 1416 (s), 1242 (s), 1052 (m), 765 (m) cm^{-1}; ^{1}H NMR (200 MHz, CDCl$_3$) δ 7.33 (m, 5 H, Ar*H*), 5.53-5.22 (AB, 2 H, CbzNCH_2O), 5.21 and 5.20 (AB, 2 H, CH_2Ph), 4.77 (br d, 1 H, 44 Hz ($^2J_{HF}$), C*H*F), 4.38 (t, 1 H, 8 Hz, CbzNC*H*CO), 3.93 (d, 1 H, 3.2 Hz, iPrC*H*N), 3.70 (s, 3 H, OCH_3), 3.68 (s, 3 H, OCH_3), 2.28 (m, 1 H, MeC*H*Me), 2.23-1.85 (m, 4 H, CH_2CH_2), 1.02 (d, 3 H, 7 Hz, CH_3), 0.67 (d, 3 H, 7 Hz, CH_3); CI MS (NH$_3$) 465 (MH$^+$). Anal. calcd for C$_{23}$H$_{29}$DN$_3$O$_6$F: C, 59.54; H, 6.52; N, 9.05. Found: C, 59.94; H, 6.61; N, 8.64.

The protected β-fluoro amino acid **60** (28.1 mg, 60.6 μmol) was hydrolyzed by a procedure analogous to that described above for production of **56** to afford 7.7 mg (45 %

yield) of (2S, 3R, 6S)-[2-^{2}H]-3-fluoro-2,6-diaminopimelic acid (**61**): IR (MeOH cast) 3600-2500 (br s), 1725 (m), 1660 (m), 1580 (m), 1498 (s), 1400 (m) cm^{-1}; ^{1}H NMR (400 MHz, D_2O, DSS) δ 4.88 (dd, 1 H, 48 Hz ($^2J_{HF}$), 10.4 Hz, C*H*F), 3.85 (t, 1 H, 6 Hz, C*H*N), 2.10-1.58 (m, 4 H, CH_2CH_2); ^{19}F NMR (376.5 MHz, D_2O) δ -191.69; FAB MS (glycerol) 210 (MH^+).

(2R, 5R)-1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-5-(1,1-difluoroprop-1-enyl) -4-imidazolidinone (62)

To a solution of **22** (R isomer, 0.520 g, 2.00 mmol) in dry THF (30 mL) at -78 °C was added dropwise LDA solution (2.03 mmol). A deep red solution resulted. Stirring was continued at -78 °C for 30 min. A solution of 3-bromo-3,3-difluoropropene[177] (0.36 g, 2.3 mmol) in dry THF (3 mL) was added dropwise. The reaction mixture was stirred at -78 °C for 40 min and then was warmed to 0 °C over 4 h. It was poured into half-saturated NH_4Cl solution. The product was extracted with ethyl ether (2 x 100 mL). The organic phase was dried over Na_2SO_4 and concentrated to offer a yellowish solid, which was purified by flash chromatography using EtOAc / hexane (30 / 70) to afford **62** in 51% yield (0.370 g). IR (CH_2Cl_2 cast) 2968 (m), 1747 (s), 1704 (s), 1632 (s), 1449 (m), 1365 (s), 1260 (m), 1187 (m), 801 (m), 716 (m) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.70-7.40 (m, 5 H, ArH), 5.67 (s, 1 H, NCHN), 4.42 (d, 1 H, J=3.2 Hz, NCHCO), 3.75 (dtd, 1 H, J=24.8, 7, 2 Hz, $CH{=}CF_2$), 3.09 (s, 3 H, NCH_3), 2.57-2.40 (m, 1 H, $CH_2CH{=}CF_2$), 1.96-1.65 (m, 1 H, $CH_2CH{=}CF_2$), 1.03 (s, 9 H, $(CH_3)_3C$); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ 170.88 (C=O), 170.54 (C=O), 156.88 (dd, J=286.8 Hz, 292.1 Hz, $CH{=}CF_2$), 136.08 (C=C), 131.93 (C=C), 128.97 (C=C), 127.75 (C=C), 80.25 (NCHN), 71.22 (dd, J=20.4, 25.7 Hz, $CH{=}CF_2$), 60.41 (NCHCO), 41.00 ($CH_2CH{=}CF_2$), 31.95 (NCH_3), 26.35 ($(CH_3)_3C$), 24.77 (s, $(CH_3)C$); CI-MS 337 (M^+H) (337.17 calcd for $C_{18}H_{23}N_2O_2F_2$); Anal.calcd for $C_{18}H_{22}N_2O_2F_2$: C, 64.29; H, 6.55; N, 8.33. Found: C, 64.17; H, 6.58; N, 8.29.

D-Glutamic Acid (63) from 62

Compound **62** (54 mg, 0.16 mmol) was dissolved in 6N HCl (10 mL) and the solution was heated to reflux for 8 h. It was cooled to 20 °C and washed with CH_2Cl_2 (20 mL). The aqueous phase was concentrated *in vacuo* to dryness. The residue was then applied to a small ion exchange column (AG50-X8, H^+ form, 1 mL bed volume). After being washed with water (30 mL), the column was eluted with 1N NH_4OH (30 mL). The solution was concentrated *in vacuo* to give **63** (11.3 mg, yield: 48%). IR (KBr) 3600-2500 (br, s), 1618-1586 (s), 1523 (s), 1403 (s) cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 3.74 (dd, 1 H, J=7.2; 5.2 Hz, OCC*H*N), 2.41-2.29 (m, 2 H, C*H*$_2$COO), 2.20-2.00 (m, 2 H, C*H*$_2$CH$_2$); FAB-MS 147.89 (MH^+) (147.05 calcd for $C_5H_9NO_4$).

(2R, 5R)-1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-5-allyl-4-imidazolidinone (64)

To a solution of **22** (R isomer, 1.04 g, 4.00 mmol) in dry THF (60 mL) at -78 °C was added dropwise LDA solution (4.20 mmol). A deep red solution resulted. Stirring was continued at -78 °C for 30 min. A solution of allyl bromide (0.610 g, 5.00 mmol) in dry THF (6 mL) was added dropwise. The reaction mixture was allowed to warm to 0 °C over 2 h. The resulting yellowish solution was poured into half-saturated NH_4Cl solution (60 mL) and the product was extracted with Et_2O (2 x 100 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo* to give a pale yellow solid, which was purified by flash chromatography using EtOAc / hexane (50 / 50) to afford a pure **64** (0.910 g, yield: 76%). IR (CH_2Cl_2 cast) 2978 (s), 1695 (s), 1640 (s), 1496 (s), 1398 (s), 1365 (s), 1260 (s), 925 (s), 721 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.72-7.40 (m, 5 H, Ar*H*), 5.62 (s, 1 H, NC*H*N), 5.45-5.20 (m, 1 H, C*H*=CH$_2$), 5.03 (d, 1 H, J = 9.2 Hz, CH=C*H*$_2$), 4.83 (d, 1 H, J=16 Hz, CH=C*H*$_2$), 4.40 (d, 1 H, J=4.5 Hz, NC*H*CO), 3.09 (s, 3 H, C*H*$_3$N), 2.64- 2.45 (m, 1 H, C*H*$_2$CH=CH$_2$), 1.96-1.66 (m, 1 H, C*H*$_2$CH=CH$_2$), 1.06 (s, 9 H, (C*H*$_3$)$_3$C); CI-MS 301 (MH$^+$) (300.18 calcd for $C_{18}H_{24}N_2O_2$); Anal. calcd for $C_{18}H_{24}N_2O_2$: C, 72.07; H, 8.06; N, 9.34. Found: C, 71.81; H, 8.22; N, 9.27.

(3R, 6S)-3,6-Dihydro-3-(1-methylethyl)-6-(3,3-difluoroprop-2-enyl)-2,5-dimethoxypyrazine (65) and (3R, 6R)-3,6-Dihydro-3-(1-methylethyl)-6-(3,3-difluoroprop-2-enyl)-2,5-dimethoxypyrazine (66)

To a stirred solution of the bislactim ether (3R isomer, 4.85 g, 26.4 mmol) in THF (90 mL) at -78 °C, was added *n*-BuLi (27.6 mL, 0.956 M, 26.39 mmol). The mixture was stirred for 10 min at this temperature and treated dropwise with a solution of 3-bromo-3,3-difluoropropene (4.34 g, 27.7 mmol) in THF (20 mL). After 5 h the reaction temperature was allowed to rise to 20 °C gradually within 6 h. The solvent was removed *in vacuo*, the residue was taken up in ether (200 mL) and washed with water (200 mL). The ether phase was dried over Na_2SO_4 and concentrated *in vacuo* to afford a liquid, which was purified by flash chromatography using EtOAc / hexane (10 / 90) to give compound **65** (4.11 g, 60% yield) and compound **66** (1.40 g, 20% yield). Compound **65**: IR ($CHCl_3$ cast) 2947 (s), 1748 (s), 1695 (s), 1463 (s), 1438 (s), 1383 (m), 1307 (s), 1241 (s), 1196 (m), 1015 (s) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.08 (dtd, 1 H, J=3, 8, 25.2 Hz; *H*C=CF$_2$), 4.12-3.87 (m, 2 H, iPrC*H*N and NC*H*C=N), 3.69 (s, 3 H, OC*H*$_3$), 3.67 (s, 3 H, OC*H*$_3$), 2.54-2.15 (m, 3 H, C*H*$_2$CHCF$_2$ and CH$_3$C*H*CH$_3$), 1.05 (d, 3 H, J=6.8 Hz, C*H*$_3$CH), 0.69 (d, 3 H, J=6.8 Hz, CHC*H*$_3$); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ 164.28 (C=N), 162.68 (C=N), 156.97 (dd, J = 285.3; 288.3 Hz, CH=*C*F$_2$), 73.62 (dd, J=20.6, 23.7 Hz, *C*H=CF$_2$), 60.94 (iPr*C*HN), 54.91 (N*C*HC=N), 52.40 (br s, O*C*H$_3$), 31.64 (Me*C*HMe), 27.57 (br s, *C*H$_2$CHCF$_2$), 19.00 (*C*H$_3$CH), 16.58 CH*C*H$_3$); MS 260.1331 (260.1336 calcd for $C_{12}H_{18}N_2O_2F_2$); Anal. calcd for $C_{12}H_{18}N_2O_2F_2$: C, 55.38; H, 6.92; N, 10.77. Found: C, 55.63; H, 7.03; N, 10.52.

Compound **66**: IR ($CHCl_3$ cast) 2948 (s), 1748 (s), 1696 (s), 1463 (m), 1437 (m), 1308 (m), 1240 (s), 1197 (m), 1016 (s) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.23 (dtd, 1 H, J=2.6; 8; 24.8 Hz, C*H*=CF_2), 4.04 (m, 1 H, iPrC*H*N), 3.95 (m, 1 H, NC*H*C=N), 3.69 (s, 3 H, OC*H*3), 3.67 (s, 3 H, OC*H*3), 2.65-2.47 (m, 1 H, MeC*H*Me), 2.40-2.17 (m, 2 H, C*H*2CH=CF_2), 1.08 (d, 3 H, J=7.5 Hz, C*H*3CH), 0.71 (d, 3 H, J=7.5 Hz, CHC*H*3); ^{13}C NMR (50 32 MHz, $CDCl_3$) δ 163.74 (C=N), 162.27 (C=N), 156.97 (dd, J = 285.3, 288.3 Hz, CH=*C*F2), 74.73 (dd, J = 20.1, 23.6 Hz, *C*H=CF_2), 60.75 (iPr*C*HN), 55.26 (N*C*HC=N), 52.34 (OCH_3), 52.27 (OCH_3), 30.99 (Me*C*HMe), 28.21 (d, J = 5 Hz, *C*H2CH=CF_2), 19.48 (*C*H3CH), 17.16 CH*C*H3); MS 260.1335 (260.1336 calcd for $C_{12}H_{18}N_2O_2F_2$); Anal.calcd for $C_{12}H_{18}N_2O_2F_2$: C, 55.38; H, 6.92; N, 10.77. Found: C, 54.91; H, 6.80; N, 10.51.

Methyl (2S)-2-Benzyloxycarbonylamino-5,5-difluoro-4-pentenoate (67) and Methyl N-Benzyloxycarbonyl-L-valinate (67a)

A mixture of compound **65** (0.45 g, 1.73 mmol) and 0.25N HCl (20 mL, 5.0 mmol) was stirred at room temperature for 4 h. It was then washed with ether (40 mL). To the aqueous solution was added $NaHCO_3$ (3.00 g, 35.7 mmol) and benzyloxycarbonyl chloride (0.800 g, 4.7 mmol) was introduced dropwise. The mixture was stirred at 20 °C for 6 h and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to give an oil, which was purified by MPLC using EtOAc / hexane (15 / 85) to give **67** (0.134 g, 26% yield): IR (CH_2Cl_2 cast) 3340 (m), 2960 (m), 1748 (s), 1725 (s), 1529 (m), 1215 (s), 1057 (m) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.34 (m, 5 H, Ar*H*), 5.39 (d, 1 H, J=7.2 Hz, N*H*), 5.12 (s, 2 H, C*H*2Ph), 4.45 (dd, 1 H, J=7.2; 5.4 Hz, OCC*H*N), 4.12 (dtd, 1 H, J=23.2; 8; 2 Hz, CH=CF_2), 3.75 (s, 3 H, C*H*3O), 2.68-2.35 (m, 2 H, C*H*2CH=CF_2); MS 299.0973 (299.0969 calcd for $C_{14}H_{15}NO_4F_2$); Anal. calcd for $C_{14}H_{15}NO_4F_2$: C, 56.24; H, 5.06; N, 4.68. Found: C, 56.59; H, 5.25; N, 4.78.

Compound 67a was isolated in 76% yield (0.348 g): IR (CH_2Cl_2 cast) 3350 (m), 2953 (m), 1743 (s), 1725 (s), 1527 (s), 1268 (m), 1220 (s), 1027 (m), 705 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.36 (m, 5 H, Ar*H*), 5.27 (d, 1 H, J=8 Hz, N*H*), 5.13 (s, 2 H, C*H*$_2$Ph), 4.32 (dd, 1 H, J=8; 4.5 Hz, OCC*H*N), 3.75 (s, 3 H, OC*H*$_3$), 2.17 (m, 1 H, MeC*H*Me), 0.97 (d, 3 H, J=7 Hz, C*H*$_3$CH), 0.90 (d, 3 H, J=7 Hz, CHC*H*$_3$); MS 265.1316 (265.1314 calcd for $C_{14}H_{19}NO_4$); Anal. calcd for $C_{14}H_{19}NO_4$: C, 63.40; H, 7.17; N, 5.28. Found: C, 63.28; H, 7.22; N, 5.31.

(1R, 2S)-2-Phenylcyclohexyl 2-Bromoacetate (68)

The literature procedure was modified.[185] To a solution of (1R, 2S)-trans-2-phenylcyclohexanol[160,161] (10.0 g, 56.8 mmol) and bromoacetic acid (20.0 g, 144 mmol) in benzene (140 mL) was added *p*-toluenesulfonic acid (0.28 g). The solution was heated to reflux in a Dean-Stark apparatus to azeotropically remove water for 21 h. After cooling, it was poured into saturated $NaHCO_3$ (250 mL). The organic layer was separated and the aqueous layer extracted with ether (3 x 150 mL). The combined organic layers were washed with brine (2 x 75 mL), dried over Na_2SO_4, then concentrated *in vacuo* to give an oil (16.8 g, yield: 99%). IR ($CHCl_3$ cast) 3030 (m), 2935 (s), 2859 (s), 1732 (s), 1494 (m), 1449 (s), 1279 (s), 1167 (s), 1108 (s), 1013 (s), 756 (s), 700 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.32-7.12 (m, 5 H, ArH), 5.01 (td, 1 H, J=10.4; 5 Hz, OC*H*), 3.52 (AB, 2 H, J=12 Hz, BrC*H*$_2$CO), 2.68 (td, 1 H, J=10.4; 4 Hz, PhC*H*), 2.22-1.23 (m, 8 H, C*H*$_2$C*H*$_2$C*H*$_2$C*H*$_2$); MS 296.0410 (296.0412 calcd for $C_{14}H_{17}O_2Br$); Anal. calcd for $C_{14}H_{17}O_2Br$: C, 56.58; H, 5.73; Br, 26.91. Found: C, 56.61; H, 5.83; Br, 26.65.

(1R, 2S)-2-Phenylcyclohexyl 2-Nitrooxyacetate (69)

The literature procedure was modified.[185] To a solution of **68** (16.5 g, 55.8 mmol) in dry CH_3CN (130 mL) was added $AgNO_3$ (25.0 g, 147 mmol). The mixture was stirred at 20 °C for 23 h, additional $AgNO_3$ (2.80 g, 16.5 mmol) was added, and stirring was continued for a further 21 h. The green solids were filtered. The filtrate was concentrated *in vacuo* to give a white solid, which was dissolved in ether, washed with water (2 x 100 mL), dried over Na_2SO_4, and concentrated *in vacuo* to afford a white solid **69** (15.3 g, yield: 98%). IR ($CHCl_3$ cast) 2950 (s), 1749 (s), 1667 (s), 1410 (m), 1292 (s), 1227 (s), 1065 (m), 849 (s), 752 (s), 700 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.34-7.09 (m, 5 H, Ar*H*), 5.06 (td, 1 H, J=10.4, 4.4 Hz, OC*H*), 4.53 (AB, 2 H, J=16 Hz, $O_2NOC\mathit{H}_2CO$, 2.67 (td, 1 H, J=11, 4 Hz, PhC*H*), 2.20-1.20 (m, 8 H, $C\mathit{H}_2C\mathit{H}_2C\mathit{H}_2C\mathit{H}_2$); MS 279.1100 (279.1107 calcd for $C_{14}H_{17}NO_5$); Anal. calcd for $C_{14}H_{17}NO_5$: C, 60.22; H, 6.09; N, 5.02. Found: C, 60.04; H, 6.10; N, 4.84.

(1R, 2S)-2-Phenylcyclohexyl Glyoxylate (70)

The literature procedure was modified.[185] Compound **69** (5.57 g, 19.9 mmol) and sodium acetate (1.65 g, 20.1 mmol) were dissolved in DMSO (70 mL). The resulting solution was stirred at 20 °C for 3 h. The solution was poured into ice/salt water (100 mL). The product was extracted with ether (3 x 100 mL) and the combined ether extracts were washed with brine (300 mL). The organic phase was dried over Na_2SO_4 and concentrated *in vacuo* to give a yellow gum, which was distilled at 100-110 °C / 0.025 mmHg (oil bath temp. 130 °C) to give **70** as a viscous oil (3.50 g, yield: 76%). IR (CH_2Cl_2 cast) 3490 (m), 2935 (s), 1748 (s), 1450 (m), 1285 (m), 1216 (s), 1122 (s), 1008 (s), 755 (m), 700 (s), 530 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.23 (s, 1 H, C*H*O), 7.44-7.04 (m, 5 H, Ar*H*), 5.22 (td, 1 H, J=10, 4 Hz, OCH), 2.88 (td, 1 H, J=11.5, 4 Hz, PhC*H*), 2.32-1.25 (m, 8 H, $C\mathit{H}_2C\mathit{H}_2C\mathit{H}_2C\mathit{H}_2$); CI-MS 232, 250 ($M^+ + H_2O$) (232.11 calcd for $C_{14}H_{16}O_3$).

Methyl (2R)-2-Amino-4-pentenoate (71)

To a solution of D-allylglycine (1.00 g, 8.69 mmol) in dry MeOH (50 mL) at 0 °C was added anhydrous HCl until saturation was reached (0.5 h). The reaction solution was brought to 20 °C and stirred overnight. The solvent was removed *in vacuo* to give **71** as an oil in quantitative yield (1.59 g). IR ($CHCl_3$ cast) 3300-2400 (br, s), 1747 (s), 1585 (m), 1500 (m), 1440 (m), 1243 (s), 930 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.68 (br s, NH_3), 5.87 (m, 1 H, $CH{=}CH_2$), 5.41-5.22 (m, 2 H, $CH{=}CH_2$), 4.30 (dt, 1 H, J=5, 5.2 Hz, OCCHN), 3.84 (s, 3 H, OCH_3), 2.87 (t, 2 H, J=6 Hz, $CH_2CH{=}CH_2$); MS 129.0790 (129.0790 calcd for $C_6H_{11}NO_2$); Anal. calcd. for $C_6H_{11}NO_2$: C, 55.80; H, 8.58; N, 10.84. Found: C, 55.45; H, 8.39; N, 10.68.

Methyl (2R)-2-(N-bromoacetylamino)-4-pentenoate (72)

D-Allylglycine methyl ester (**71**) (1.43 g, 8.64 mmol) was dissolved in a mixture of $CHCl_3$ (30 mL) and 5% $NaHCO_3$ (60 mL). To this was added bromoacetyl chloride (1.88 g, 95% purity, 11.3 mmol). The mixture was stirred at 20 °C for 5 h. The $CHCl_3$ layer was washed with 5% $NaHCO_3$ (60 mL), dried over Na_2SO_4, and concentrated *in vacuo* to give **72** as a white amorphous solid (1.95 g, yield: 90%). IR (CH_2Cl_2 cast) 3329 (s), 2955 (s), 1737 (s), 1645 (s), 1544 (s), 1441 (s), 1363 (s), 1346 (s), 1266 (s), 1237 (s), 1153 (s), 993 (s), 931 (s), 630 (s), 601 (s), 522 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.00 (br s, 1 H, NH), 5.80-5.57 (m, 1 H, $CH{=}CH_2$), 5.24-5.10 (m, 2 H, $CH{=}CH_2$), 4.65 (dt, 1 H, J=8, 4.8 Hz, OCCHN), 3.90 (s, 2 H, $BrCH_2CO$), 3.77 (s, 3 H, OCH_3), 2.72-2.47 (m, 2 H, $CH_2CH{=}CH_2$); MS 248.9987 (249.0000 calcd for $C_8H_{12}NO_3Br$); Anal. calcd for $C_8H_{12}NO_3Br$: C, 38.42; H, 4.84; N, 5.60. Found: C, 38.53; H, 4.84; N, 5.58.

Methyl (2R)-2-(N-nitrooxyacetylamino)-4-pentenoate (73)

To a solution of N-bromoacetyl-D-allylglycine methyl ester (**72**) (1.85 g, 7.40 mmol) in CH_3CN (20 mL) was added $AgNO_3$ (3.80 g, 22.4 mmol). The mixture was stirred at 20 °C for 24 h, after which an additional 2.44 g $AgNO_3$ (14.4 mmol) was added to the solution, and stirring was continued for another 24 h. The green solids (AgBr) were filtered. The filtrate was concentrated *in vacuo* to give a liquid, which was dissolved in ether (100 mL), washed with water (100 mL), dried over Na_2SO_4, and concentrated *in vacuo* to afford **73** as an oil (0.903 g, yield: 52%). IR (CH_2Cl_2 cast) 3310 (m), 2960 (m), 1746 (s), 1653 (s), 1545 (m), 1440 (m), 1280 (s), 1225 (m), 849 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 6.57 (br s, 1 H, N*H*), 5.77-5.54 (m, 1 H, C*H*=CH_2), 5.24-5.07 (m, 2 H, CH=CH_2), 4.93 (AB, 2 H, OCCH_2ONO_2), 4.74 (dt, 1 H, J=8; 5 Hz, OCC*H*N), 3.78 (s, 3 H, CH_3O), 2.73-2.45 (m, 2 H,CH_2CH=CH_2); MS 232.0696 (232.0695 calcd for $C_8H_{12}N_2O_6$).

Methyl (2S)-2-(N-bromoacetylamino)-5,5-difluoro-4-pentenoate (74)

A mixture of **65** (1.74 g, 6.69 mmol) and 0.25N HCl (80 mL, 22.0 mmol) was stirred at 20 °C for 6 h. The solution was washed with ether (2 x 30 mL). To the aqueous phase were added $NaHCO_3$ (5 g) and $CHCl_3$ (30 mL). Then bromoacetyl chloride (2.74 g, 17.4 mmol) was introduced dropwise. The resulting mixture was stirred at 20 °C for overnight. The $CHCl_3$ phase was washed with 5% aqueous $NaHCO_3$ (30 mL), dried over Na_2SO_4, and concentrated *in vacuo* to give an oil. This was purified by MPLC using EtOAc / hexane (25 / 75) to give pure **74** in 74% yield (1.17 g). IR ($CHCl_3$ cast) 3300 (m), 1748 (s), 1662 (s), 1540 (m), 1210 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.04 (br s, 1 H, *H*N), 4.66 (dt, 1 H, J=7; 6 Hz, OCC*H*N), 4.14 (dtd, 1 H, J=24; 8; 2 Hz, C*H*=CF_2), 3.90 (s, 2 H, BrCH_2CO), 3.79 (s, 3 H, OCH_3), 2.74-2.40 (m, 2 H, CH_2CH=CF_2); MS 284.9806 (284.9812 calcd for $C_8H_{10}NO_3F_2Br$); Anal. calcd for $C_8H_{10}NO_3F_2Br$: C, 33.73; H, 3.54; N, 4.91. Found: C, 33.85; H, 3.67; N, 4.89.

Methyl (2S)-2-(N-Nitrooxyacetylamino)-5,5-difluoro-4-pentenoate (75)

To a solution of **74** (0.141 g, 0.493 mmol) in CH_3CN (5 mL) was added $AgNO_3$ (0.252 g, 1.48 mmol). The mixture was stirred at 20 °C for 60 h. The green solids (AgBr) were filtered by gravity filtration. The filtrate was concentrated *in vacuo* to give a liquid, which was dissolved in ether (10 mL), washed with water (20 mL), dried over Na_2SO_4, and concentrated *in vacuo* to afford **75** as an oil in 93% yield (0.123 g). IR ($CHCl_3$ cast) 3320 (m), 1749 (s), 1656 (s), 1548 (m), 1280 (s), 1241 (m), 848 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 6.68 (d, 1 H, J=6 Hz, N*H*), 4.93 (AB, 2 H, J=16 Hz, OCCH_2ONO$_2$), 4.73 (dt, 1 H, J=8, 5.6 Hz, OCC*H*N), 4.10 (dtd, 1 H, J=24, 8, 2 Hz, C*H*=CF$_2$), 3.80 (s, 3 H, OCH_3), 2.74-2.40 (m, 2 H, CH_2CH=CF$_2$); MS 268.0493 (268.0507 calcd for $C_8H_{10}N_2O_6F_2$); Anal. calcd for $C_8H_{10}N_2O_6F_2$: C, 35.83; H, 3.76; N, 10.45. Found: C, 35.89; H, 3.89; N, 9.95.

(2S)-2-(N-Benzyloxycarbonylamino)-5-[5,7-bis-(1,1-dimethylethyl) benzoxazol-2-yl]pentanoic Acid (76)

Triethylamine (1.0 mL, 7.2 mmol) was added to a suspension of N_α-CBz-L-lysine (0.446 g, 1.6 mmol) in dry MeOH (25 mL) containing 3, 5-di-*tert*-butyl-o-benzoquinone (0.35 g, 1.6 mmol). The mixture was stirred at 20 °C for 1.5 h during which it became homogeneous and green. Aqueous 1N HCl (20 mL) was added at 0 °C, and the product was extracted into EtOAc (3 x 50 mL). The organic solution was dried over Na_2SO_4 and concentrated *in vacuo* to give a pink solid (hygroscopic), which was purified by flash chromatography using EtOAc (0.5% HCO_2H) to give pure **76** (0.486 g) in 63.6% yield. IR ($CHCl_3$ cast) 3320 (br s), 2960 (s), 2560 (br m) 1725 (s), 1575 (s), 1525 (s), 1405 (s), 1230 (s), 1155 (m), 1060 (s), 755 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.72 (br s, 1H, COO*H*), 7.53 (d, 1H, J=1.5 Hz, tert-BuAr*H*), 7.41-7.21 (m, 5H, OCH$_2$Ar*H*), 7.26 (d, 1H, J=1.5 Hz, tert-BuAr*H*), 5.88 (d, 1H, J=8 Hz, *H*N), 5.11 (s, 2H, CH_2Ph), 4.51 (br d, 1H, J=8 Hz, OCC*H*N), 3.02 (br t, 2H, N=CCH_2), 2.15-1.85 (m, 4H, CH_2CH_2), 1.46 (s, 9H, (CH_3)$_3$C), 1.34 (s, 9H, (CH_3)$_3$C); CI-MS 481 (MH^+) (480.26 calcd for $C_{28}H_{36}N_2O_5$).

1,3,5-Tribenzylhexahydro 1,3,5-triazine (77)

The literature procedure was modified.[212] Benzylamine (15.7 g, 0.146 mol) was dissolved in water (150 mL) at 20 °C. To the solution was added formaldehyde (37% aqueous, 14.3 g, 0.176 mol) and the resulting mixture was stirred at 20 °C overnight. The product was extracted with EtOAc (2 x 200 mL), the combined organic phases were dried over Na_2SO_4, and the solution was concentrated *in vacuo* to afford **77** as an oil (17.5 g, yield: 99%). IR (CH_2Cl_2 cast) 3020 (m), 2810 (m), 1490 (m), 1450 (m), 1360 (m), 1168 (s), 915 (m), 738 (s), 898 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.36-7.15 (m, 15 H, Ar*H*), 3.64 (s, 6 H, PhCH_2), 3.36 (br s, 6 H, NCH_2N); MS 357.2199 (357.2205 calcd for $C_{24}H_{27}N_3$); Anal. calcd for $C_{24}H_{27}N_3$: C, 80.67; H, 7.56; N, 11.76. Found: C, 80.31; H, 7.58; N, 11.86.

Diethyl N-Benzylaminomethylphosphonate (78)

The literature procedure was modified.[213] A mixture of 1,3,5-tribenzylhexahydro-1,3,5-triazine (**77**) (17.5 g, 48.9 mmol) and diethylphosphite (20.2 g, 147 mmol) was heated at 100 °C for 8 h. The reaction mixture was then partitioned between water (400 mL) and EtOAc (400 mL). The aqueous phase was extracted with EtOAc (400 mL). The combined EtOAc solutions were dried over Na_2SO_4 and concentrated *in vacuo* to give a liquid, which was purified by distillation under vacuum (140 °C / 0.01 mmHg) to give **78** (24.1 g, 64% yield). IR (CH_2Cl_2 cast) 3310 (m), 2980 (s), 1455 (m), 1243 (s), 1058 (s), 1028 (s), 963 (s), 740 (m), 700 (m), 540 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.40-7.20 (m, 5 H, Ar*H*), 4.15 (p, 4 H, J=7.2 Hz, OCH_2CH$_3$), 3.88 (s, 2 H, CH_2Ph), 2.95 (d, 2 H, J_{HP}=12 Hz, NCH_2P), 1.67 (br s, 1 H, N*H*), 1.34 (t, 6 H, J=7.2 Hz, OCH$_2$CH_3); MS 257.1178 (257.1181 calcd for $C_{12}H_{20}NO_3P$); Anal. calcd for $C_{12}H_{20}NO_3P$: C, 56.03; H, 7.78; N, 5.45. Found: C, 56.43; H, 7.85; N, 5.44.

Diethyl Aminomethylphosphonate (79)

The literature procedure was modified.[213] A mixture of diethyl N-benzylaminomethylphosphonate (**78**) (23.5 g, 91.5 mmol) and 10% palladium on charcoal (4.0 g) in EtOH (200 mL) was hydrogenated at 20 °C and atmospheric pressure overnight. The mixture was filtered through Celite and the filtrate was concentrated *in vacuo* to give a liquid, which was distilled under vacuum (85 °C / 0.5 mmHg) to give **79** (14.4 g, 94% yield). IR (CH_2Cl_2 cast) 3340 (m, doublet), 2980 (m), 1296 (s), 1055 (s), 1028 (s), 962 (s), 770 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.17 (p, 4 H, J=7.2 Hz, OCH_2CH_3), 3.02 (d, 2 H, J=10 Hz, NCH_2P), 1.36 (t, 6 H, J=7.2 Hz, OCH_2CH_3); MS 167.0711 (167.0711 calcd for $C_5H_{14}NO_3P$); Anal. calcd for $C_5H_{14}NO_3P$: C, 35.93; H, 8.38; N, 8.38. Found: C, 36.30; H, 8.48; N, 8.23.

Diethyl (1S, 4R)-(1,7,7-Trimethylbicyclo[2.2.1]hept-2-ylidene)aminomethyl -phosphonate (80)

The literature procedure was modified.[200] A mixture of diethyl aminomethylphosphonate (**79**) (6.00 g, 35.9 mmol), 1S (-) camphor (6.00 g, 39.5 mmol) and a catalytic amount of $BF_3 \cdot OEt_2$ in dry benzene (100 mL) was heated at reflux for 20 h with water removal by a Dean-Stark apparatus. The solvent was removed *in vacuo*. The residue was purified by flash chromatography using EtOAc to remove excess camphor, followed by EtOAc / MeOH (95 / 5). The product was purified by distillation (115 °C / 0.01 mmHg) to give **80** (4.45 g, 41% yield). IR (CH_2Cl_2 cast) 2958 (s), 1682 (s), 1445 (m), 1390 (m), 1243 (s), 1059 (s), 1035 (s), 964 (s), 775 (m), 520 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.16 (p, 4 H, J=7.2 Hz, 2 OCH_2CH_3), 3.80 (dd, 2 H, J=17, 1.6 Hz, NCH_2P), 2.54-2.37 (m, 1 H, $CHCH_2$), 2.00-1.60 (m, 4 H, CH_2CH_2), 1.45-1.15 (m, 2 H, $CHCH_2$), 1.34 (t, 6 H, J=7.2 Hz, OCH_2CH_3), 0.98 (s, 3 H, CH_3), 0.94 (s, 3 H, CH_3), 0.80 (s, 3 H, CH_3); MS 301.1814 (301.1807 calcd for $C_{15}H_{28}NO_3P$); Anal. calcd for $C_{15}H_{28}NO_3P$: C, 59.78; H, 9.36; N, 4.64. Found: C, 59.58; H, 9.59; N, 4.71.

Diethyl (1R, 4S)-(1,7,7-Trimethylbicyclo[2.2.1]hept-2-ylid)eneaminomethyl -phosphonate (81)

The literature procedure was modified.[200] D-(+) camphor (6.05 g, 39.8 mmol) was converted to the corresponding chiral imine by the procedures analogous to the preparation of imine **80**. Compound **81** was obtained in 38% yield (4.16 g). IR (CH_2Cl_2 cast) 2959 (s), 1682 (s), 1445 (m), 1390 (s), 1243 (s), 1060 (s), 1037 (s), 963 (s), 775 (m), 530 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.15 (p, 4 H, J=7.2 Hz, OCH_2CH_3), 3.79 (dd, 2 H, J=17, 1.6 Hz, NC*H*P), 2.54-2.35 (m, 1 H, $CHCH_2$), 2.00-1.58 (m, 4 H, CH_2CH_2), 1.45-1.14 (m, 2 H, $CHCH_2$), 1.33 (t, 6 H, J=7.2 Hz, OCH_2CH_3), 0.96 (s, 3 H, CH_3), 0.92 (s, 3 H, CH_3), 0.79 (s, 3 H, CH_3); MS 301.1804 (301.1807 calcd for $C_{15}H_{28}NO_3P$); Anal. calcd for $C_{15}H_{28}NO_3P$: C, 59.78; H, 9.36; N, 4.64. Found: C, 59.39; H, 9.48; N, 4.57.

(1S, 2S, 5S)-2-Hydroxy-2,6,6-trimethylbicyclo[3.1.1]heptan-3-one (82)

The literature procedure was modified.[214] To a cold (ice bath) solution of (+) α-pinene (15.0 g, 0.110 mol) in 90% aqueous acetone (163 g) was added with stirring potassium permanganate (30.0 g, 0.190 mol) over a period of 2 h. The reaction mixture was stirred at 5 °C for an additional 38 h, filtered, evaporated to a volume of 40 mL, and then extracted with ether (3 x 200 mL). The combined ethereal extracts were washed with water and 5% aqueous $NaHCO_3$ (400 mL), dried over Na_2SO_4, and concentrated to give an oil. This was distilled (115 °C / 17 mmHg) to give the ketol **82** in 40% yield (7.36 g). IR (CH_2Cl_2 cast) 3440 (s), 2929 (s), 1720 (s), 1474 (s), 1408 (m), 1371 (s), 1162 (s), 1077 (s), 1051 (s), 919 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.64 (br t, 2 H, $OCCH_2$), 2.55-2.40 (m, 1 H, CH_2CHCH_2), 2.26 (s, 1 H, OH), 2.14 (d, 2 H, J=6.5 Hz, $CHCH_2CH$), 1.70 (d, 1 H, J=11 Hz, $CHCH_2$), 1.40 (s, 3 H, CH_3), 1.38 (s, 3 H, CH_3), 0.91 (s, 3 H, CH_3); MS 168.1151 (168.1150 calcd for $C_{10}H_{16}O_2$).

Diethyl (1S, 2S, 5S)-(2-Hydroxy-2,6,6-trimethylbicyclo[3.1.1]hept-3-ylidene)aminomethyl phosphonate (83)

The literature procedure was modified.[201] The ketol **82** (3.88 g, 23.1 mmol) was transformed to the corresponding chiral imine **83** in 82% yield (3.29 g), by procedures analogous to the preparation of imine **80**. The final product was purified by flash chromatography using EtOAC / MeOH (95 / 5) instead of by distillation because it tended to decompose at high temperature (> 140 °C). IR ($CHCl_3$ cast) 3370 (m), 2981 (s), 2916 (s), 1720 (m), 1650 (m), 1370 (m), 1240 (s), 1162 (m), 1054 (s),1031 (s), 968 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.18 (pd, 4 H, J=8, 2.4 Hz, $OC\mathit{H}_2CH_3$), 3.92 (d, 2 H, J=17.5 Hz, $NC\mathit{H}_2P$), 3.00 (br s, 1 H, OH), 2.62 (br t, 2 H, $C\mathit{H}_2C{=}N$), 2.44-2.28 (m, 1 H, $CH_2C\mathit{H}CH_2$), 2.06 (d, 2 H, J=6 Hz, $CHC\mathit{H}_2CH$), 1.55 (d, 1 H, J=11.2 Hz, $C\mathit{H}CH_2$), 1.50 (s, 3 H, $C\mathit{H}_3$), 1.35 (td, 6 H, J=8; 2.4 Hz, $OCH_2C\mathit{H}_3$), 1.33 (s, 3 H, $C\mathit{H}_3$), 0.89 (s, 3 H, $C\mathit{H}_3$); ^{31}P NMR (81.0 MHz, $CDCl_3$, H_3PO_4) (^1H decoupled) δ 23.17 (s); MS 317.1744 (317.1756 calcd for $C_{15}H_{28}NO_4P$).

(2R, 5R)-1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-5-(3-bromopropyl)-4-imidazolidinone (84)

The alkylation of (2R)-1-benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinone (**22**) (4.50 g, 17.3 mmol) with dibromopropane (3.87 g, 14.2 mmol) by the procedure analogous to the preparation of compound **85** below, produced compound **84** in 66% yield (4.37 g): IR (CH_2Cl_2 cast) 2970 (m), 1707 (s), 1646 (s), 1364 (m), 1260 (m), 715 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.70-7.40 (m, 5 H, ArH), 5.65 (s, 1 H, $^tBuC\mathit{H}N$), 4.38 (br d, 1 H, J=4 Hz, $OCC\mathit{H}NCO$), 3.09 (s, 3 H, $NC\mathit{H}_3$), 3.15-2.92 (m, 2 H, $BrC\mathit{H}_2$), 1.93-0.91 (m, 4 H, $C\mathit{H}_2C\mathit{H}_2$), 1.06 (s, 9 H, $C(CCH_3)_3$); MS-CI 381 ($M^+{+}1$) (380.1 calcd for $C_{18}H_{25}N_2O_2Br$); Anal. calcd for $C_{18}H_{25}N_2O_2Br$: C, 56.70; H, 6.61; N, 7.34. Found: C, 57.12; H, 6.65; N, 7.26.

(2S, 5S)-1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-5-(3-bromopropyl)-4-imidazolidinone (85)

To a solution of (2S)-1-benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinone (**23**) (1.50 g, 5.77 mmol) in anhydrous THF (90 mL) at -78 °C, was added freshly prepared LDA solution (1.15 eq). The reaction mixture was stirred at -78 °C for 25 min before the addition of dibromopropane (1.29 g, 6.39 mmol) in THF (10 mL). After being stirred at -78 °C for 1 h, the reaction mixture was gradually warmed to 20 °C over 6 h, and then stirred at 20 °C overnight. The reaction mixture was poured into half-saturated aqueous NH_4Cl solution (40 mL). The aqueous phase was extracted with EtOAc (3 x 80 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo* to give an oil, which was purified by flash chromatography using EtOAc/hexane (50/50). Compound **85** was obtained a pure amorphous solid in 57% yield (1.25 g). IR (CH_2Cl_2 cast) 2960 (m), 1707 (s), 1640 (s), 1360 (m), 1255 (m), 710 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.70-7.40 (m, 5 H, Ar*H*), 5.65 (s, 1 H, ^{t}BuC*H*N), 4.38 (br d, 1 H, J=4 Hz, OCC*H*NCO), 3.10 (s, 3 H, NCH_3), 3.15-2.92 (m, 2 H, $BrCH_2$), 1.92-0.92 (m, 4 H, CH_2CH_2), 1.08 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ 171.3 (s, C=O), 137.1 (s, C=C), 132.2 (s, C=C), 129.3 (s, C=C), 128.0 (s, C=C), 80.3 (s, N*C*HN), 60.8 (s, N*C*HCO), 41.4 (s, CH_3N), 32.2 (s, CH_2Br), 32.0 (s, $CH_2CH_2CH_2Br$), 30.0 (s, CH_2CH_2Br), 26.5 (s, $(CH_3)_3C$), 26.2 (s, $(CH_3)_3C$); MS 323.0392 (M^+-$C(CH_3)_3$) (323.0396 calcd for $C_{14}H_{16}N_2O_2Br$); Anal. calcd for $C_{18}H_{25}N_2O_2Br$: C, 56.70; H, 6.61; N, 7.34; O, 8.39. Found: C, 56.80; H, 6.65; N, 7.19; O, 8.57.

Diethyl [(2S, 5S, 1'S, 4'R, 1"R)-4-[1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinon-5-yl]-1-(1,7,7-trimethylbicyclo[2.2.1]hept-2-ylideneamino)butyl-phosphonate (86) and Diethyl [(2S, 5S, 1'S, 4'R, 1"S)

4-[1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinon-5-yl]-1-(1,7,7-trimethylbicyclo[2.2.1]hept-2-ylideneamino)butylphosphonate (87)

To a solution of the chiral imine **80** (0.530 g, 1.76 mmol) in THF (10 mL) at -78 °C, was added dropwise a *n*-BuLi solution (1.20 M, 1.69 mL, 2.03 mmol). The resulting solution was stirred at -78 °C for 25 min before addition of bromide **85** (0.671 g, 1.76 mmol) in THF (5 mL). The solution was stirred at -78 °C for 1 h, then warmed to 20 °C gradually over 6 h and stirred at 20 °C overnight. The reaction mixture was poured into half-saturated aqueous NH_4Cl solution (20 mL). The aqueous phase was extracted with EtOAc (3 x 50 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo* to afford an oil, which was purified by flash chromatography using EtOAc/MeOH (95/5) to give a mixture of **86** and **87** (0.752 g, 71% yield for the mixture) in a 4:1 ratio.

Compound **86** (0.529 g, 50% yield)was separated from **87** (0.110 g, 10% yield) on an MPLC column (silica gel, 2.5 x 30 cm) using EtOAc as eluent. For **86**: IR (CH_2Cl_2 cast) 2958 (s), 1709 (s), 1680 (m), 1650 (s), 1366 (m), 1258 (m), 1055 (m), 1029 (s), 981 (m), 710 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.65-7.38 (m, 5 H, Ar*H*), 5.58 (s, 1 H, ^{t}BuC*H*N), 4.31 (br d, 1 H, J=4 Hz, OCCHNCO) 4.08 (m, 4 H, 2 C$H_2$$CH_3$), 3.46 (ddd, 1 H, J=4, 9, 12 Hz, PC*H*N), 3.05 (s, 3 H, HCH_3), 2.56-2.40 (m, 1 H, C*H*CH_2), 2.00-1.16 (m, 12 H, CH_2CH_2 and CHCH_2), 1.26 (dt, 6 H, J_{HH}=7.2 Hz, J_{HP}=4.8 Hz, 2 OCH_2CH_3), 1.05 (s, 9 H, C(CH_3)$_3$), 1.00 (s, 3 H, CH_3), 0.94 (s, 3 H, CH_3), 0.85 (s, 3 H, CH_3); ^{31}P NMR (81.0 MHz, $CDCl_3$, H_3PO_4) (^1H decoupled) δ 24.97 (s); CI-MS 602 (MH^+) (601.4 calcd for $C_{33}H_{52}N_3O_5P$); Anal. calcd for $C_{33}H_{52}N_3O_5P$: C, 65.87; H, 8.71; N, 6.98. Found: C, 65.73; H, 8.86; N, 6.95.

For **87**: IR (CH_2Cl_2 cast) 2960 (s), 1710 (s), 1680 (m), 1649 (s), 1368 (m), 1260 (m), 1057 (m), 1027 (s), 989 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.65-7.38 (m, 5 H, Ar*H*), 5.54 (s, 1 H, ^{t}BuC*H*), 4.31 (br s, 1 H, OCC*H*NCO), 4.24-3.92 (m, 4 H, 2 OC$H_2$$CH_3$), 3.48 (ddd, 1 H, J=4, 9.5, 14 Hz, OPC*H*N), 3.00 (s, 3 H, NCH_3), 2.28-

1.14 (m, 13 H, CH_2CH_2 and $CHCH_2$), 1.29 (dt, 6H, J_{HH}=7 Hz, J_{HP}=2.5 Hz, 2 $POCH_2CH_3$), 1.04 (s, 9 H, $C(CH_3)_3$), 0.93 (s, 3 H, CH_3), 0.90 (s, 3 H, CH_3), 0.71 (s, 3 H, CH_3); ^{31}P NMR (81.0 MHz, $CDCl_3$, H_3PO_4) (^{1}H decoupled) δ 24.43 (s); CI-MS 602 (MH$^+$) (601.4 calcd for $C_{33}H_{52}N_3O_5P$). Anal. calcd for $C_{33}H_{52}N_3O_5P$: C, 65.87; H, 8.71; N, 6.98. Found: C, 65.59; H, 8.69; N, 6.92.

Diethyl [(2R, 5R, 1'S, 4'R, 1"R)-4-[1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinon-5-yl]-1-(1,7,7-trimethylbicyclo[2.2.1]hept-2-ylideneamino)butyl-phosphonate (88) and Diethyl [(2R, 5R, 1'S, 4'R, 1"S)-4-[1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinon-5-yl]-1-(1,7,7-trimethylbicyclo[2.2.1]hept-2-ylideneamino)butylphosphonate (89)

Alkylation of the chiral imine **80** (2.00 g, 6.64 mmol) with the bromide **84** (2.53 g, 6.64 mmol) by the procedure analogous to the preparation of compounds **86** and **87**, afforded a mixture of compounds **88** and **89** in 86% yield (3.43 g) in a ratio of 3:2.

Compound **88** (1.25 g, 31% yield) was separated from **89** (1.03 g, 26%) on an MPLC column (silica gel, 2.5 x 30 cm) using EtOAc as eluent. For **88**: IR (CH_2Cl_2 cast) 2958 (s), 1709 (s), 1680 (m), 1647 (s), 1366 (m), 1260 (m), 1254 (s), 1028 (s), 965 (m) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.65-7.37 (m, 5 H, ArH), 5.57 (s, 1 H, ^{t}BuCHN), 4.30 (br s, 1 H, OCCHNCO), 4.22-3.93 (m, 4 H, OCH_2CH_3), 3.48 (ddd, 1 H, J=4.8, 8.8, 12.8 Hz, PCHN), 3.04 (s, 3 H, NCH_3), 2.54-2.38 (m, 1 H, CHCH), 1.99-1.11 (m, 12 H, CH_2CH_2 and $CHCH_2$), 1.26 (dt, 6 H, J_{HH}=8 Hz, J_{HP}=4.4 Hz, $POCH_2CH_3$), 1.04 (s, 9 H, $C(CH_3)_3$), 0.90 (s, 6 H, 2 CH_3), 0.83 (s, 3 H, CH_3); ^{31}P NMR (81.0 MHz, $CDCl_3$, H_3PO_4) (^{1}H decoupled) δ 24.79 (s); CI-MS 602 (MH$^+$) (601.4 calcd for $C_{33}H_{52}N_3O_5P$). Anal. calcd for $C_{33}H_{52}N_3O_5P$: C, 65.87; H, 8.71; N, 6.98. Found: C, 65.91; H, 8.82; N, 6.89.

For **89**: IR (CH_2Cl_2 cast) 2958 (s), 1711 (s), 1680 (s), 1649 (s), 1448 (s), 1366 (s), 1259 (s), 1055 (s), 1027 (s), 962 (s), 714 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.65-7.37 (m, 5 H, Ar*H*), 5.49 (s, 1 H, *tert*-BuC*H*N), 4.30 (br s, 1 H, OCC*H*NCO), 4.22-3.95 (m, 4 H, OC$H_2$$CH_3$), 3.45 (ddd, 1 H, J=2.8, 11.2, 16 Hz, PC*H*N), 3.01 (s, 3 H, NCH_3), 2.54-1.11 (m, 13 H, CH_2CH_2 and CHCH_2), 1.29 (dt, 6 H, J_{HH}=8 Hz, J_{HP}=5.2 Hz), 1.09 (s, 3 H, CH_3), 1.05 (s, 9 H, C(CH_3)$_3$), 0.98 (s, 3 H, CH_3), 0.80 (s, 3 H, CH_3); ^{31}P NMR (81.0 MHz, $CDCl_3$, H_3PO_4) (^1H decoupled) δ 24.47 (s); CI-MS 602 (MH^+) (601.4 calcd for $C_{33}H_{52}N_3O_5P$); Anal. Calcd for $C_{33}H_{52}N_3O_5P$: C, 65.87; H, 8.71; N, 6.98. Found: C, 65.70; H, 8.79; N, 6.96.

Diethyl [(2S, 5S, 1'R, 4'S, 1"S)-4-[1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinon-5-yl]-1-(1,7,7-trimethylbicyclo[2.2.1]hept-2-ylideneamino)butyl-phosphonate (90) and Diethyl [(2S, 5S, 1'R, 4'S, 1"R)-4-[1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinon-5-yl]-1-(1,7,7-trimethylbicyclo[2.2.1]hept-2-ylideneamino)butylphosphonate (91)

Alkylation of the chiral imine **81** (2.00 g, 6.64 mmol) with bromide **85** (2.53 g, 6.64 mmol) by the procedure analogous to the preparation of compounds **86** and **87**, afforded a mixture of compound **90** and **91** in 88% yield (3.52 g) in a ratio of 3:2. Compound **90** (1.31 g, 33% yield) was separated from **91** (0.906 g, 23% yield) on an MPLC column (silica gel, 2.5 x 30 cm) using EtOAc as eluent.

For **90**: IR ($CHCl_3$ cast) 2958 (s), 1710 (s), 1647 (s), 1367 (m), 1259 (m), 1055 (m), 1027 (m), 962 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.65-7.37 (m, 5 H, Ar*H*), 5.57 (s, 1 H, NC*H*N), 4.30 (br s, 1 H, OCC*H*N), 4.22-3.93 (m, 4 H, OC$H_2$$CH_3$), 3.48 (ddd, 1 H, J=4.8, 8.8, 12.8 Hz, PC*H*N), 3.04 (s, 3 H, NCH_3), 2.54-2.38 (m, 1 H, C*H*CH_2), 1.99-1.11 (m, 12 H, CH_2CH_2 and CHCH_2), 1.26 (dt, 6 H, J_{HH}=8 Hz, J_{HP}=4.4 Hz, OCH_2CH_3), 1.04 (s, 9 H, (CH_3)$_3$C), 0.90 (s, 6 H, 2 CH_3), 0.83 (s, 3 H, CH_3); ^{31}P NMR (81.0 MHz, $CDCl_3$, H_3PO_4) (^{1}H decoupled) δ 24.79 (s); CI-MS 602 (MH^+) (601.3645 calcd for $C_{33}H_{52}N_3O_5P$); Anal. calcd for $C_{33}H_{52}N_3O_5P$: C, 65.87; H, 8.71; N, 6.98. Found: C, 65.57; H, 8.75; N, 6.97.

For **91**: IR ($CHCl_3$ cast) 2960 (s), 1709 (s), 1679 (m), 1648 (s), 1365 (s), 1258 (m), 1055 (s), 1027 (s), 9.62 (m) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.65-7.37 (m, 5 H, Ar*H*), 5.49 (s, 1 H, NC*H*N), 4.30 (br d, 1 H, J=4 Hz, NC*H*CO), 4.22-3.95 (m, 4 H, OCH_2CH$_3$), 3.45 (ddd, 1 H, J=2.8, 11.2, 16 Hz, PC*H*N), 3.01 (s, 3 H, NCH_3), 2.54-1.11 (m, 13 H, CH_2CH_2 and CHCH_2), 1.29 (dt, 6 H, J_{HH}=8 Hz, J_{HP}=5.2 Hz, OCH$_2$CH_3), 1.09 (s, 3 H, CH_3), 1.05 (s, 9 H, (CH_3)$_3$C), 0.98 (s, 3 H, CH_3), 0.80 (s, 3 H, CH_3); ^{31}P NMR (81.0 MHz, $CDCl_3$, H_3PO_4) (^{1}H decoupled) δ 24.49 (s); CI-MS 602 (601.3645 calcd for $C_{33}H_{52}N_3O_5P$); Anal. calcd for $C_{33}H_{52}N_3O_5P$: C, 65.87; H, 8.71; N, 6.98. Found: C, 65.92; H, 8.60; N, 6.83.

(1R, 5S)-1,5-Diamino-5-carboxypentylphosphonic Acid (92)

Compound **86** (0.220 g, 0.366 mmol) was dissolved in 12 N aqueous HCl (10 mL) and stirred at 20 °C for 1 day. This was then heated at reflux for 3 days. The solvent was removed *in vacuo* and the residue was applied to a cellulose column (2.5 x 22 cm). The product was eluted with nBuOH/H_2O/CH_3CO_2H (4/2/1) to give 95.0 mg of **92** as the dihydrochloride salt (yield: 87%). For **92**: IR (MeOH cast) 3600-2400 (br s), 1728 (s), 1595 (s), 1504 (s), 1300-850 (br s) cm^{-1}; ^{1}H NMR (200 MHz, D_2O, DSS) δ 4.14 (t, 1 H, J=6 Hz, OCC*H*N), 3.35 (dt, 1 H, J_{HH}=6 Hz, J_{HP}=13 Hz, OPC*H*N), 2.15-1.54 (m, 6 H, CH_2CH_2CH_2); ^{13}C NMR (50.32 MHz, D_2O) δ 171.3 (s, C=O), 52.1 (s, OC*C*HN), 48.1 (d, J_{PC}=144.3 Hz, OP*C*HN), 29.0 (s, *C*H$_2$CHNCOOH), 27.6 (s, *C*H$_2$CHNPO), 21.0 (d, J=9.2 Hz, CH$_2$*C*H$_2$CH$_2$); ^{31}P NMR (81.0 MHz, D_2O, H_3PO_4) (^{1}H decoupled) δ 14.03 (s); FAB-MS 227 (MH^+) (226.07 calcd for $C_6H_{15}N_2O_5P$).

(1S, 5S)-1,5-diamino-5-carboxyl pentylphosphonic acid (93)

Compound **87** (80 mg, 0.133 mmol) was hydrolyzed in 12 N aqueous HCl to afford **93** as its dihydrochloride salt (35 mg, yield: 88%). The hydrolysis conditions and subsequent purification procedures were analogous to those used for conversion of compound **86** to **92**. For **93**: IR (MeOH cast) 3600-2400 (br s), 1730 (m), 1590 (m), 1510 (m), 1300-850 (br s) cm^{-1}; ^{1}H NMR (200 MHz, D_2O, DSS) δ 4.14 (t, 1 H, J=6.4 Hz, OCC*H*N), 3.34 (dt, 1 H, J_{HH}=6 Hz, J_{HP}=13 Hz, OPC*H*N), 2.15-1.54 (m, 6 H, $CH_2CH_2CH_2$); ^{13}C NMR (50.32 MHz, D_2O) δ 171.3 (s, C=O), 52.0 (s, OC*C*HN), 48.1 (d, J_{PC}=144.3 Hz, OP*C*HN), 28.8 (s, CH_2CHNCOOH), 27.4 (s, CH_2CHNPO), 20.9 (d, J=7.4 Hz, $CH_2CH_2CH_2$); ^{31}P NMR (81.0 MHz, D_2O, H_3PO_4) (^{1}H decoupled) δ 14.21 (s); FAB-MS 227 (MH^+) (226.07 calcd for $C_6H_{15}N_2O_5P$).

(1R, 5R)-1,5-Diamino-5-carboxypentylphosphonic Acid (94)

Compound **88** (0.225 g, 0.374 mmol) was hydrolyzed in 12 N aqueous HCl to give **94** as its dihydrochloride salt (82.5 mg, yield: 74%). The hydrolysis conditions and subsequent purification procedure were analogous to those used for conversion of compound **86** to **92**. For **94**: IR (MeOH cast) 3600-2400 (br s), 1730 (m), 1590 (m), 1510 (m), 1300-850 (br s) cm^{-1}; ^{1}H NMR (200 MHz, D_2O, DSS) δ 4.14 (t, 1 H, J=6.4 Hz, OCC*H*N), 3.34 (dt, 1 H, J_{HH}=6 Hz, J_{HP}=14 Hz, OPC*H*N), 2.15-1.56 (m, 6 H, $CH_2CH_2CH_2$); ^{13}C NMR (50.32 MHz, D_2O) δ 171.8 (s, C=O), 52.5 (s, OC*C*HN), 48.5 (d, J_{PC} = 144.9 Hz, OP*C*HN), 29.4 (s, CH_2CHNCOOH), 27.9 (s, CH_2CHNPO), 21.4 (d, J = 8 Hz, $CH_2CH_2CH_2$); ^{31}P NMR (81.0 MHz, D_2O, H_3PO_4) (^{1}H decoupled) δ 14.21 (s); FAB-MS 227 (MH^+) (226.07 calcd for $C_6H_{15}N_2O_5P$).

(1S, 5R)-1,5-Diamino-5-carboxypentylphosphonic Acid (95)

Compound **89** (0.155 g, 0.258 mmol) was hydrolyzed in 12 N aqueous HCl to afford **95** as its dihydrochloride salt (54.0 mg, yield: 70%). The procedures for hydrolysis and subsequent purification were analogous to those used for conversion of compound **86** to

92. For **95**: IR (KBr) 3700-2400 (br s), 1730 (m), 1610 (m), 1520 (m), 1300-850 (br m) cm^{-1}; ^{1}H NMR (200 MHz, D_2O, DSS) δ 4.14 (t, 1 H, J=6.4 Hz, OCC*H*N), 3.34 (dt, 1 H, J_{HH}=6 Hz, J_{HP}=14 Hz, OPC*H*N), 2.15-1.55 (m, 6 H, $C\textit{H}_2C\textit{H}_2C\textit{H}_2$); ^{13}C NMR (50.32 MHz, D_2O) δ 171.9 (s, C=O), 52.6 (s, OC*C*HN), 48.5 (d, J_{PC}=144.4 Hz, OP*C*HN), 29.4 (s, $\textit{C}H_2CHNCOOH$), 28.0 (s, $\textit{C}H_2CHNPO$), 21.4 (d, J = 7.5 Hz, $CH_2\textit{C}H_2CH_2$); ^{31}P NMR (81.0 MHz, D_2O, H_3PO_4) (^{1}H decoupled) δ 13.88 (s); FAB-MS 227 (MH$^+$) (226.07 calcd for $C_6H_{15}N_2O_5P$).

Tetraethyl (1S, 4R, 1'S, 4'R)-1,5-bis(1,7,7-Trimethylbicyclo[2.2.1]hept-2-ylideneamino)pentane-1,5-diyl diphosphonate (96)

To a solution of the chiral imine **80** (0.709 g, 2.36 mmol) in THF (15 mL) at -78 °C, was added dropwise *n*-BuLi solution (2.59 mmol). Stirring was continued at -78 °C for 20 min. After addition of 1,3-dibromopropane (0.240 g, 1.19 mmol) in THF (4 mL), the reaction mixture was stirred at -78 °C for 3 h, then warmed to 20 °C over 2 h and stirred overnight. The mixture was poured into half-saturated aqueous NH_4Cl (15 mL). The product was extracted with EtOAc (3 x 60 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo* to give an oil, which was purified by flash chromatography using EtOAc/MeOH (95/5) to afford pure **96** as a mixture of all possible isomers at the α-centers in 62% yield (0.472 g): IR (CH_2Cl_2 cast) 2958 (s), 1680 (s), 1445 (m), 1390 (m), 1246 (s), 1056 (s), 1028 (s), 962 (s), 577 (s) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.26-3.98 (m, 8 H, $O\textit{CH}_2CH_3$), 3.72-3.50 (m, 2 H, NC*H*PO), 2.64-1.00 (m, 20 H, $C\textit{H}_2C\textit{H}_2$ and $C\textit{H}C\textit{H}_2$), 1.40-1.22 (m, 12H, 4 $OCH_2C\textit{H}_3$), 0.96 (s, 6H, 2 $C\textit{H}_3$), 0.92 (s, 6H, 2 $C\textit{H}_3$), 0.86 (s, 3H, $C\textit{H}_3$), 0.72 (s, 3 H, $C\textit{H}_3$); ^{31}P NMR (81.0 MHz, $CDCl_3$, H_3PO_4) δ 25.25 (s) and 25.01 (s) in ratio of 7:3; MS 642.3930 (642.3893 calcd for $C_{33}H_{60}N_2O_6P_2$); Anal. calcd. for $C_{33}H_{60}N_2O_6P_2$: C, 61.66; H, 9.41; N, 4.36. Found: C, 61.40; H, 9.25; N, 4.20.

Tetraethyl (1R, 4S, 1'R, 4'S)-1,5-bis(1,7,7-Trimethylbicyclo[2.2.1]hept-2-ylideneamino)pentane-1,5-diyl diphosphonate (97)

The alkylation of chiral imine **81** (0.683 g, 2.27 mmol) with 1, 3-dibromopropane (0.230 g, 1.14 mmol) was accomplished to afford the desired compound **97** (0.474 g) in 65% yield. The alkylation procedure was analogous to the one used to synthesize the compound **96** as described above. The spectroscopic properties of **97** were identical to those of **96**.

1,5-Diaminopentane-1,5-diyl Diphosphonic Acid (98)

Compound **96** (0.201 g, 0.312 mmol) was dissolved in 12 N aqueous HCl (8 mL) and The solution was stirred at 20 °C for 1 day. The solution was then heated to reflux for 3 days. The solvent was removed *in vacuo*, the residue was applied to a cellulose column (2.5 x 27 cm), which was eluted with nBuOH/H_2O/CH_3CO_2H (4/2/1). The desired compound **98** was obtained as a dihydrochloride salt (a mixture of isomers) in 63% yield (65.4 mg): IR (MeOH cast) 3700-2000 (br s), 1615 (s), 1520 (s), 1170 (s), 1060 (s), 940 (s) cm^{-1}; ^{1}H NMR (200 MHz, D_2O, DSS) δ 3.38 (dt, 2 H, J_{HH}=6.5 Hz, J_{HP}=14 Hz, 2 OPC*H*N), 2.15-1.57 (m, 6 H, C*H*$_2$C*H*$_2$C*H*$_2$); ^{13}C NMR (50.32 MHz, D_2O) δ 48.41 (d, J=145.4 Hz, OP*C*HN), 48.28 (d, J=145.0 Hz, OP*C*HN), 27.98 (s, PCHN*C*H$_2$), 27.88 (s, PCHN*C*H$_2$), 22.44 (t, J=8.6 Hz, CH$_2$*C*H$_2$CH$_2$), 22.29 (t, J=7.4 Hz, CH$_2$*C*H$_2$CH$_2$); ^{31}P NMR (81.0 MHz, D_2O, H_3PO_4) δ 14.57 (s); FAB-MS 263 (MH^+) (262.04 calcd for $C_5H_{16}N_2O_6P_2$).

(4S)-2,5-Dioxo-4-methyloxazolidine (99)

The literature procedure was modified.[149] To a stirred suspension of L-alanine (2.00 g, 22.5 mmol) in THF (30 mL) at 20 °C was added a stream of phosgene for 40 min. The solution was heated at 50 °C overnight, during which time it became clear. The excess phosgene was removed by bubbling Ar through the solution and trapping the gas into a 1 N aqueous NaOH solution. The solvent was evaporated *in vacuo* and the residue was dissolved in THF. The process was repeated twice to remove hydrogen chloride. A solid product was obtained, which was dissolved in EtOAc (6 mL) and crystallization was induced by adding hexane (6 mL). After filtration, the material was dried under vacuum to give **99** (1.40 g, yield: 54%), mp 92 °C (lit.[149] 91-92 °C); IR (KBr) 3342 (br s), 1857 (s), 1769 (s), 1283 (s), 1140 (m), 925 (s), 756 (m), 590 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 6.53 (br s, 1 H, *H*N), 4.40 (q, 1 H, J=6.8 Hz, C*H*NCO), 1.58 (d, 3 H, J=6.8 Hz, C*H*3CHN); CI-MS 133 ($M^+ + H_2O$) (115.027 calcd for $C_4H_5NO_3$); Anal. calcd for $C_4H_5NO_3$: C, 41.74; H, 4.35; N, 12.17. Found: C, 41.98; H, 4.37; N, 12.29.

(1R, 5S)-N_5-L-Alanyl-1,5-diamino-5-carboxypentylphosphonic Acid (100)

To a stirred solution of **92** (26 mg, 0.115 mmol) in 1N aqueous Na_2CO_3 (0.5 mL) at 20 °C was added simultaneously dropwise the solution of L-alanine N-carboxy anhydride **99** (26 mg, 0.226 mmol) in dry THF (2 mL) and 1 N aqueous NaOH (0.5 mL). The reaction mixture was then stirred at 20 °C for 10 min. The solvent was removed *in vacuo*, and the residue was applied to a cellulose column (2.5 x 27 cm). The desired product was eluted with nBuOH/H_2O/CH_3CO_2H (4/2/1) to give **100** (16 mg, yield: 47%). IR (MeOH cast) 3600-2400 (br, s), 1670 (s), 1580 (br, s), 1395 (m), 1160-1065 (m) cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 4.17 (t, 1 H, J=8.8 Hz, NC*H*CO_2H), 4.10 (q, 1 H, J=7.2 Hz, CH_3C*H*CON), 3.21 (m, 1 H, NC*H*PO_3H_2), 2.04-1.30 (m, 6 H, C*H*2C*H*2C*H*2), 1.54 (d, 3 H, J=7.2 Hz, C*H*3C); ^{13}C NMR (50.32 MHz, D_2O) δ 178.40 (s, O=CO), 170.15 (O=CN), 55.38 (s, *C*HCOOH), 49.30 (d, J = 135.9 Hz, $CHPO_3H$), 49.10 (s, N*C*HCH_3), 31.08 (s, *C*H2CHCOOH), 28.40 (s, *C*H2CHPO_3H), 22.36 (d, J=4.0 Hz, CH_2*C*H2CH_2), 16.45 (s, *C*H3CH); FAB-MS 298 (MH^+) (297.11 calcd. for $C_9H_{20}N_3O_6P$).

(1S, 5S)-N_5-L-Alanyl-1,5-diamino-5-carboxypentylphosphonic Acid (101)

The isomer **93** (47.0 mg, 0.208 mmol) was acylated with L-alanine N-carboxy anhydride **99** (51.0 mg, 0.443 mmol) by using procedures analogous to those employed for the conversion of **92** to the dipeptide **100**. The desired dipeptide **101** was obtained in 23% yield (14.0 mg). IR (MeOH cast) 3600-2400 (br, s), 1671 (s), 1580-1540 (s), 1390 (m), 1160-1060 (s) cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 4.22 (dd, 1 H, J_1=5.2 Hz, J_2=8 Hz, $CH_2C\mathit{H}CO_2H$), 4.14 (q, 1 H, J=7.2 Hz, $CH_3C\mathit{H}NH_2$), 3.27 (m, 1 H, $CH_2C\mathit{H}PO_3H$), 2.10-1.30 (m, 6 H, $C\mathit{H}_2C\mathit{H}_2C\mathit{H}_2$), 1.60 (d, 3 H, J=7.2 Hz, $C\mathit{H}_3C$); ^{13}C NMR (50.32 MHz, D_2O) δ 178.35 (s, O=CO), 170.16 (s, O=CN), 55.07 (s, $\mathit{C}HCOOH$), 49.09 (d, J=142.4 Hz, $CHPO_3H$), 49.09 (s, $N\mathit{C}HCH_3$), 30.90 (s, $\mathit{C}H_2CHCOOH$), 28.18 (s, $\mathit{C}H_2CHPO_3H$), 22.21 (d, J=8.1 Hz, $CH_2\mathit{C}H_2CH_2$), 16.42 (s, $\mathit{C}H_3CH$); FAB-MS 298 (MH^+) (297.11 calcd. for $C_9H_{20}N_3O_6P$).

(1S, 5R)-N_5-L-Alanyl-1,5-diamino-5-carboxypentylphosphonic Acid (102)

The isomer **95** (54.0 mg, 0.239 mmol) was acylated with L-alanine N-carboxy anhydride **99** (57.0 mg, 0.496 mmol) by using procedures analogous to those employed for the conversion of **92** to the dipeptide **100**. The desired dipeptide **102** was obtained in 19% yield (14.2 mg). IR (MeOH cast) 3600-2400 (br, s), 1671 (s), 1585 (s), 1540 (s), 1390 (m), 1165-1060 (m) cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 4.22 (dd, 1 H, J_1=5.2 Hz, J_2=8 Hz, $CH_2C\mathit{H}CO_2H$), 4.15 (q, 1 H, J=7.2 Hz, $CH_3C\mathit{H}NH_2$), 3.27 (m, 1 H, $CH_2C\mathit{H}PO_3H$), 2.10-1.30 (m, 6 H, $C\mathit{H}_2C\mathit{H}_2C\mathit{H}_2$), 1.62 (d, 3 H, J=7.2 Hz, $C\mathit{H}_3CH$); ^{13}C NMR (50.32 MHz, D_2O) δ 178.51 (s, O=CO), 170.41 (s, O=CN), 55.28 (s, $\mathit{C}HCO_2H$), 49.15 (d, J=140.9 Hz, $\mathit{C}HPO_3H$), 49.24 (s, $N\mathit{C}HCH_3$), 31.08 (s, $\mathit{C}H_2CHCOOH$), 28.32 (s, $\mathit{C}H_2CHPO_3H$), 22.50 (d, J=9.6 Hz, $CH_2\mathit{C}H_2CH_2$), 16.68 (s, $\mathit{C}H_3CH$); FAB-MS 298 (MH^+) (297.11 calcd. for $C_9H_{20}N_3O_6P$).

(1R, 5R)-N_5-L-Alanyl-1,5-diamino-5-carboxypentylphosphonic Acid (103)

The isomer **94** (40.0 mg, 0.177 mmol) was acylated with L-alanine N-carboxy anhydride **99** (41.0 mg, 0.356 mmol) by using procedures analogous to those employed for the conversion of **92** to the dipeptide **100**. The desired dipeptide **103** was obtained in 19% yield (10.0 mg). IR (MeOH cast) 3600-2400 (br, s), 1670 (s), 1580 (br, s), 1400 (m), 1160-1070 (s) cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 4.21 (dd, 1 H, J_1=5.5 Hz, J_2=8 Hz, CH_2CHCO_2H), 4.14 (q, 1 H, J=7.5 Hz, CH_3CHNH_2), 3.25 (m, 1 H, CH_2CHPO_3H), 2.10-1.30 (m, 6 H, $CH_2CH_2CH_2$), 1.57 (d, 3 H, J=7.5 Hz, CH_3C); ^{13}C NMR (50.32 MHz, D_2O) δ 178.56 (s, O=*C*O), 170.37 (s, O=*C*N), 55.09 (s, *C*HCOOH), 49.12 (d, J=144.9 Hz, *C*HPO3H), 49.19 (s, N*C*HCH3), 30.69 (s, CH_2CHCO_2H), 28.22 (s, CH_2CHPO_3H), 22.31 (d, J=9.1 Hz, $CH_2CH_2CH_2$), 16.63 (s, CH_3CH); FAB-MS 298 (MH^+) (297.11 calcd. for $C_9H_{20}N_3O_6P$).

(S)-3-[3-(Benzyloxycarbonyl)-5-oxazolidinon-4-yl]propyl *p*-Toluenesulfonate (104)

The literature procedure was modified.[229] To a solution of compound **16** (1.03 g, 3.69 mmol) and dry pyridine (4.0 mL, 3.91g, 49.5 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C, *p*-toluenesulfonyl chloride (2.11 g, 11.07 mmol) in dry CH_2Cl_2 (15 mL) was added dropwise. The reaction mixture was then stirred at 0 °C for 40 h. It was quenched with cold water (40 mL). The organic phase was separated and washed with 20% $CuSO_4 \cdot 5H_2O$ solution (150 mL), 5% $NaHCO_3$ (150 mL) and water (150 mL). The organic phase was dried over Na_2SO_4 and concentrated *in vacuo* to give **104** as an oil (1.33 g, 83% yield). IR ($CHCl_3$ cast) 1800 (s), 1718 (s), 1415 (m), 1358 (s), 1175 (s), 930 (m) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, 2 H, J=9 Hz, Ar*H*), 7.80-7.64 (m, 7 H, Ar*H*), 5.52 (s, 1 H, NC*H*O), 5.26-5.10 (m, 3 H, NC*H*O and C*H*2Ph), 4.23 (t, 1 H, NC*H*CO), 4.01 (br s, 2 H, OCH_2CH_2), 2.43 (s, 3 H, CH_3Ph), 2.06-1.56 (m, 4 H, CH_2CH_2); MS 433.1183 (433.1195 calcd for $C_{21}H_{23}NO_7S$); Anal. calcd for

$C_{21}H_{23}NO_7S$: C, 58.20; H, 5.31; N, 3.23; S, 7.39. Found: C, 58.01; H, 5.51; N, 3.09; S, 7.26.

(S)-3-[3-(Benzyloxycarbonyl)-5-oxazolidinon-4-yl]propyl Iodide (105)

The literature procedure was modified.[228] To a stirred solution of magnesium metal (0.320 g, 13.2 mmol) in dry ether (15 mL) was added iodine (3.05 g, 12.0 mmol) in portions. The solution was at reflux during the addition of iodine. After 0.5 h, the brown color of iodine had disappeared and the solution became almost colorless. Compound **104** (1.20 g, 2.77 mmol) in dry THF (10 mL) was added dropwise. The mixture was then stirred at 20 °C for 6 h. It was quenched with cold water. The aqueous phase was separated and extracted with CH_2Cl_2 (3 x 40 mL). The combined organic phases were decolorized by washing with 5% $Na_2S_2O_3$ solution (50 mL), dried over Na_2SO_4, and concentrated *in vacuo* to give an oil. This was purified by flash chromatography using EtOAc / hexane (30 / 70) to afford the pure **105** (0.367 g, yield: 34%). IR ($CHCl_3$ cast) 2920 (m), 1800 (s), 1717 (s), 1415 (s), 1355 (m), 1215 (m), 1050 (m), 765 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.40 (s, 5 H, Ar*H*), 5.56 (s, 1 H, NC*H*O), 5.36-5.16 (m, 3 H, NC*H*O and C*H*$_2$Ph), 4.35 (br s, 1 H, NC*H*CO), 3.15 (br s, 2 H, IC*H*$_2$), 2.22-1.78 (m, 4 H, C*H*$_2$C*H*$_2$); MS 389.0094 (389.0124 calcd for $C_{14}H_{16}NO_4I$); Anal. calcd for $C_{14}H_{16}NO_4I$: C, 43.20; H, 4.11; N, 3.60. Found: C, 43.54; H, 3.97; N, 3.58.

(S)-3-[3-(Benzyloxycarbonyl)-5-oxazolidinon-4-yl]propyl *p*-Nitrobenzenesulfonate (106)

To a solution of compound **16** (0.455 g, 1.63 mmol) and dry pyridine (1.7 mL, 1.66 g, 21.0 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C, was added dropwise 4-nitrobenzenesulfonyl chloride (1.08 g, 4.87 mmol) in dry CH_2Cl_2 (20 mL) . The reaction mixture was then stirred at 0 °C for 32 h. Cold water (20 mL) was added to quench the reaction. The CH_2Cl_2 phase was separated and washed with 20% $CuSO_4 \cdot 5H_2O$ solution

(75 mL), water (75 mL) and 5% NaHCO$_3$ solution (75 mL). It was then dried over Na$_2$SO$_4$ and concentrated *in vacuo* to give an oil. This was purified by flash chromatography using EtOAc / hexane (40 / 60) to give a pure **106** (0.372 g, 49% yield). IR (CHCl$_3$ cast) 1800 (s), 1717 (s), 1533 (s), 1415 (m), 1353 (s), 1185(s), 930 (m), 745 (m), 610 (m) cm^{-1}; ^{1}H NMR (200 MHz, CDCl$_3$) δ 8.40 (d, 2 H, J=8 Hz, Ar*H*), 8.08 (d, 2 H, J=8 Hz, Ar*H*), 7.40 (s, 5 H, ArH), 5.53 (br s, 1 H, NC*H*O), 5.29- 5.13 (m, 3 H, NC*H*O and CH_2Ph), 4.28 (t, 1 H, J=5.2 Hz, NC*H*CO), 4.13 (br t, 2 H, OCH_2), 2.13-1.66 (m, 4 H, CH_2CH_2); MS 464.0914 (464.0890 calcd for C$_{20}$H$_{20}$N$_2$O$_9$S).

(2S, 5S, 3"S, 6"R)-5-[3-(3,6-Dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazin-6-yl)propyl]-1-benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinone (107)

The alkylation of the bislactim ether **42** (S isomer, 0.801 g, 4.35 mmol) with the bromide **85** (1.66 g, 4.36 mmol) was accomplished by employing the procedures analogous to the preparation of compound **112** to give the desired compound **107** in 30% yield (0.635 g). IR (CH$_2$Cl$_2$ cast) 2960 (m), 1709 (s), 1649 (s), 1364 (m), 1238 (s) cm^{-1}; ^{1}H NMR (200 MHz, CDCl$_3$) δ 7.69-7.39 (m, 5 H, Ar*H*), 5.62 (s, 1 H, NC*H*N), 4.33 (br d, 1 H, OCC*H*N), 3.91 (t, 1 H, J=4 Hz, *i*PrC*H*N), 3.81 (dt, 1 H, J=6, 4 Hz, NC*H*C=N), 3.73 (s, 3 H, OCH_3), 3.65 (s, 3 H, OCH_3), 3.06 (s, 3 H, CH_3N), 2.25 (pd, 1 H, J = 7.2; 4 Hz, MeC*H*Me), 1.85- 1.63 (m, 1 H, CH_2CH$_2$), 1.50-1.23 (m, 2 H, CH$_2$CH_2), 1.17-0.64 (m, 3 H, CH_2CH_2), 1.05 (s, 9 H, (CH_3)$_3$C), 0.98 (d, 3 H, J=11 Hz, CH_3CH), 0.69 (d, 3 H, J=7.2 Hz, CHCH_3); ^{13}C NMR (50.32 MHz, CDCl$_3$) δ 171.31 (C=O), 163.57 (C=N), 136.73 (C=C), 131.43 (C=C), 128.75 (C=C), 127.39 (C=C), 79.95 (NCHN), 61.34 (iPr*C*HN), 60.76 (N*C*HCO), 54.69 (N*C*HC=N), 52.27 (OCH$_3$), 40.91 (CH$_3$N), 33.63 (Me*C*HMe), 31.76 (CH$_2$CH$_2$*C*H$_2$), 30.30 (*C*H$_2$CH$_2$CH$_2$), 26.24 ((*C*H$_3$)$_3$C), 16.97 (CH$_2$*C*H$_2$CH$_2$), 17.24 (*C*H$_3$CH), 16.58 (CH*C*H$_3$); CI-MS 485 (MH$^+$)

(484.3050 calcd for $C_{27}H_{40}N_4O_4$); Anal. calcd. for $C_{27}H_{40}N_4O_4$: C, 66.92; H, 8.32; N, 11.56. Found: C, 66.66; H, 8.11; N, 11.37.

(2R, 6S)-Diaminopimelic acid (108)

Compound **107** (50.8 mg, 0.105 mmol) was hydrolyzed to meso-diaminopimelic acid **108** in 60% yield (12 mg), by employing procedures analogous to the preparation of compound **113** described below. IR (MeOH cast) 3500-2400 (br, s), 1735 (s), 1660 (m), 1585 (m), 1490 (s), 1200-1105 (s) cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 4.08 (t, 2 H, J=6 Hz, 2 OCC*H*N), 2.20-1.90 (m, 4 H, $CH_2CH_2CH_2$), 1.84-1.48 (m, 2 H, $CH_2CH_2CH_2$); ^{13}C NMR (50.32 MHz, D_2O) δ 172.21 (C=O), 52.78 (OC*C*HN), 29.35 ($CH_2CH_2CH_2$), 20.35 ($CH_2CH_2CH_2$); FAB-MS 191.22 (MH^+) (190.0954 calcd for $C_7H_{14}N_2O_4$).

(2S, 5S, 4R)-5-(4-Amino-4-carbomethoxybutyl)-1-benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinone (109)

The compound **107** (0.221 g, 0.457 mmol) was selectively hydrolyzed to compound **109** in 83% yield (0.147 g) by employing procedures analogous to the preparation of compound **114** describe below. In this case, the product was further purified by flash chromatography using EtOAc / MeOH (90 / 10). IR (CH_2Cl_2 cast) 3350 (m, doublet), 2960 (m), 1736 (s), 1707 (s), 1645 (s), 1381 (s), 1259 (m), 715 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.69-7.41 (m, 5 H, Ar*H*), 5.65 (s, 1 H, NC*H*N), 4.37 (s, 1 H, NC*H*CO), 3.72 (s, 3 H, OCH_3), 3.24 (t, 1 H, J=6 Hz, H_2NCHCO), 3.10 (s, 3 H, CH_3N), 1.87-1.66 (m, 1 H, $CHHCH_2$), 1.54 (br s, 2 H, CH_2CH_2), 1.35-0.84 (m, 3 H, $CHHCH_2$), 1.07 (s, 9 H, $(CH_3)_3C$); CI-MS 390 (MH^+) (389.2315 calcd for $C_{21}H_{31}N_3O_4$); Anal. calcd. for $C_{21}H_{31}N_3O_4$: C, 64.76; H, 8.02; N, 10.79. Found: C, 64.57; H, 7.88; N, 10.71.

(S)-8-Benzoyl-7-(1,1-dimethylethyl)-6-methyl-6,8-diazaspiro[3.4]octan-5-one (111)

Compound **111** (65.3 mg, 5% yield) was isolated as a side-product from the alkylation of **42** with **85** described above. IR ($CHCl_3$ cast) 2950 (s), 1701 (s), 1643 (s), 1450 (m), 1352 (s), 1308 (m), 725 (m) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.66-7.40 (m, 5 H, Ar*H*), 5.60 (s, 1 H, NC*H*N), 3.06 (s, 3 H, $NC\textit{H}_3$), 2.86-2.47 (m, 2 H, $C\textit{H}_2CH_2CH_2$), 2.24 (t, 2 H, J=8 Hz, $CH_2CH_2C\textit{H}_2$), 2.07-1.82 (m, 1 H, $CH_2C\textit{H}_2CH_2$), 1.50-1.23 (m, 1 H, $CH_2C\textit{H}_2CH_2$), 0.96 (s, 9 H, $(C\textit{H}_3)_3C$); CI-MS 301 (MH$^+$) (300.1838 calcd for $C_{18}H_{24}N_2O_2$).

(2R, 5R, 3"S, 6"R)-5-[(3,6-Dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazin-6-yl)-propyl]-1-benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinone (112)

To a solution of the bislactim ether **42** (S isomer, 0.320 g, 1.74 mmol) in dry THF at -70 °C, was added dropwise *n*-BuLi (1.26M, 1.92 mmol). The solution was stirred at -70 °C for 20 min before the addition of the bromide **84** (0.671 g, 1.76 mmol) in THF (7 mL). The reaction temperature was allowed to rise to 20 °C gradually over 6 h, and was then stirred at this temperature overnight. The reaction mixture was quenched with half-saturated NH_4Cl solution (40 mL). The product was purified by a silica gel MPLC column (2.5 x 30 cm) using EtOAc / hexane (30 / 70) to give pure **112** (0.256 g, yield: 30%). IR (CH_2Cl_2 cast) 2960 (m), 1709 (s), 1649 (s), 1365 (m), 1238 (s) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.72-7.40 (m, 5 H, Ar*H*), 5.63 (s, 1 H, NC*H*N), 4.34 (s, 1 H, NC*H*CO), 3.91 (t, 1 H, J=4 Hz, *i*PrC*H*N), 3.80 (dt, 1 H, J=8; 4 Hz, NC*H*C=N), 3.68 S, 6 H, 2 $OC\textit{H}_3$), 3.06 (s, 3 H, $C\textit{H}_3N$), 2.24 (pd, 1 H, J=7.2; 4 Hz, MeC*H*Me), 1.88-1.46 (m, 3 H, $C\textit{H}HC\textit{H}_2$), 1.05 (s, 9 H, $(C\textit{H}_3)_3C$), 1.18-0.75 (m, 3 H, $C\textit{H}HC\textit{H}_2$), 0.97 (d, 3 H, J=12 Hz, $C\textit{H}_3CH$), 0.67 (d, 3 H, J=7.2 Hz, $CHC\textit{H}_3$); CI-MS 485 (MH$^+$) (484.3050 calcd for $C_{27}H_{40}N_4O_4$).

(2R, 6R)-Diaminopimelic Acid (113)

Compound **112** (35 mg, 0.072 mmol) was dissolved in 6N HCl (6 mL) and the solution was heated to reflux for 24 h. The solvent was removed *in vacuo* and the residue was applied to a cellulose column, which was eluted with nBuOH / H_2O / CH_3CO_2H (4 / 2/ 1). The product **113** was obtained in 68% yield (13 mg) as the dihydrochloride salt. IR (MeOH cast) 3600-2500 (br, s), 1650 (br, s), 1485 (s), 1415 (m), 1100 (br, s) cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 4.08 (t, 2 H, J=6.3 Hz, 2 OCC*H*N), 2.15-1.87 (m, 4 H, $CH_2CH_2CH_2$), 1.75-1.50 (m, 2 H, $CH_2CH_2CH_2$); ^{13}C NMR (50.32 MHz, D_2O) δ 172.05 (C=O), 52.83 (OC*C*HN), 29.58 ($CH_2CH_2CH_2$), 20.63 ($CH_2CH_2CH_2$); FAB-MS 191.18 (MH^+) (190.0954 calcd for $C_7H_{14}N_2O_4$); Anal. calcd. for $C_7H_{14}N_2O_4$: C, 44.20; H, 7.42; N, 14.73. Found: C, 44.01; H, 7.29; N, 14.62.

(2R, 5R, 4R)-5-(4-Amino-4-carbomethoxybutyl)-1-benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinone (114)

Compound (**112**) (153 mg, 0.316 mmol) was dissolved in 0.25N HCl (10 mL) and acetone (3 mL). The solution was then stirred at 20 °C overnight. It was neutralized to pH 8 with concentrated aqueous $NaHCO_3$ solution and was then extracted with EtOAc (5 x 30 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo* to afford **114** as an oil (106 mg, yield: 86%). IR (CH_2Cl_2 cast) 3350 (m, doublet), 2963 (s), 1737 (s), 1707 (s), 1645 (s), 1448 (s), 1365 (s), 1261 (s), 1174 (s), 1105 (s), 1026 (s),802 (s), 716 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.68-7.40 (m, 5 H, Ar*H*), 5.64 (s, 1 H, NC*H*N), 4.35 (s, 1 H, NC*H*CO), 3.70 (s, 3 H, OCH_3), 3.27 (dt, 1 H, J=3, 4.8 Hz, NH_2CHCO), 3.08 (s, 3 H, CH_3N), 1.86-0.76 (m, 6 H, $CH_2CH_2CH_2$), 1.05 (s, 9 H, $(CH_3C)_3C$); CI-MS 390 (MH^+) (389.2315 calcd for $C_{21}H_{31}N_3O_4$); Anal. calcd. for $C_{21}H_{31}N_3O_4$: C, 64.76; H, 8.02; N, 10.79. Found: C, 64.58; H, 7.90; N, 10.64.

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